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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.53(c).

Docket Number	00742/074001	Type a plus sign (+) inside this box --->	+

INVENTOR(S)/APPLICANT(S)

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TITLE OF THE INVENTION (280 characters max)

INHIBITORS OF THE MAP KINASE PATHWAY

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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

⊠ A check or money order is enclosed to cover the filing fees. ⊠ The Commissioner is hereby authorized to charge filing fees not covered and apply any credits to Deposit Account Number: 03-2095	FILING FEE AMOUNT	$80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

□ No.

■ Yes, the name of the U.S. Government agency and the Government contract number are: NIH CA-46595

⊠ Applicant claims small entity status under 37 C.F.R. § 1.27.

Respectfully submitted,

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PROVISIONAL APPLICATION

UNDER 37 C.F.R. § 1.53(c)

APPLICANT : John Blenis and Leon O. Murphy

TITLE : INHIBITORS OF THE MAP KINASE PATHWAY

INHIBITORS OF THE MAP KINASE PATHWAY

This invention was funded by grant CA-46595 from the National Institute of Health. The government may have certain rights in the invention.

Field of the Invention

This invention relates to the development and use of human therapeutics that inhibit intracellular signaling via the MAP kinase pathways.

Background of the Invention

The evolutionarily conserved Ras-MAPK signaling network regulates diverse biological processes such as cell proliferation, differentiation, migration, and survival. Many of the regulators and effectors within this network have been implicated in diverse pathological processes. MAP kinases and their targets have been identified as, for example, potent oncogenes or tumor suppressor genes and proinflammatory mediators.

Normally, the MAPK network is activated when growth factors or hormones bind to cell surface receptors. The extracellular signal is amplified and converted into an appropriate biological response. However, dysfunction of any component of the signaling pathway may result in a pathological condition. Cancer, for example, is a disease marked by the uncontrolled growth of abnormal cells. Cancerous cells have overcome the barriers imposed in normal cells, which have a finite lifespan, and grow indefinitely. As the growth of cancer cells continues, genetic alterations can accrue and persist so that the cancerous cell displays increasingly aggressive growth phenotypes. If left untreated, metastasis, the spread of cancer cells to distant areas of the body by way of the lymph system or bloodstream, may ensue, destroying healthy tissue and, ultimately, leading to

death. According to a recent American Cancer Society study, at least 1,268,000 new cancer cases are expected to be diagnosed in the United States in any given year.

However in cancer cells, mutations in upstream activators of MAPK, such as Ras or Raf, lead to constitutive signaling even in the absence of growth factors. Constitutively activating mutations in Ras are detected in at least 30% of all human malignancies but are present in especially high levels in colon (50%) and pancreatic cancers (90%). The activation kinetics of the ERK1/2-MAPK signaling pathway have also been associated with distinct biological outcomes. In fibroblasts, sustained ERK1/2 activation over several hours induces entry into S phase of the cell cycle while transient (20-30 min) activation does not.

In various cell types, the ERK1/2 pathway also has a critical role in regulation of nucleotide biosynthesis, transcription, migration, cell survival, differentiation and adaptive responses. Specifically, ERK signaling can control cardiomyocyte cell growth and the response to ventricular heart failure, cell survival in atherosclerosis, various metabolic processes such as glucose uptake, protein synthesis and leptin signaling, regulation of the immune response such as in T cell activation and inflammatory cytokine signaling, and mediating the effect of neurotransmitters that control memory and behavior. ERK signaling also can control the induction of genes that are required for establishing circadium rhythms.

Accordingly, small molecule drugs that can selectively inhibit regulatory proteins within the ERK1/2-MAPK pathway have enormous therapeutic potential. General MAPK inhibitors, however, are likely to be toxic due to the many metabolic and proliferative functions regulated by this pathway in healthy cells. ERK1/2 specifically recognizes some physiological substrates through the presence of ERK1/2 docking sites in substrates (Jacobs *et al.*, 1999; Tanoue *et al.*, 2000). At least two classes of docking site have been identified and are known as the D-box and DEF domain.

Substrate docking directs ERK1/2 to phosphorylate specific amino acids known to regulate the biological function of the substrate. Interaction of ERK1/2 with the D-box

docking site is required for ERK's initial activation by MEK, as well as its inactivation by phosphatases (Tanoue *et al.*, 2000). By contrast, the DEF domain appears to be mainly found in downstream targets of ERK1/2 (Jacobs et al., 1999).

Summary of the Invention

We have discovered that MAP kinases (e.g., extracellular signal-regulated kinase 1/2 (ERK1/2)), bind to certain target proteins (e.g., immediate early gene (IEG) products) through a DEF domain, causing the phosphorylation of target residues and a resulting biological effect (e.g., progression through the cell cycle). Thus, a variety of human diseases may be treated by blocking the interaction of MAP kinases with the DEF domain of target proteins.

Accordingly, in one aspect, the present invention provides for a method of identifying therapeutic compounds that affect the MAP kinase-DEF domain interaction. The method consists of the steps of: (i) providing test cells that express a target protein having a DEF domain and capable of promoting progression through the cell cycle; (ii) culturing the test cells in the presence of a growth factor, cytokine, tumor promoter, or oncogene under conditions that activate a target kinase; (iii) contacting the test cells with the candidate compound; (iv) assessing the binding of the MAP kinase to the target protein, wherein a candidate compound that inhibits the binding is a therapeutic compound. Desirably, the test cells are mammalian; most desirably human. Suitable test cells include, for example, a primary cell line, an immortalized cell line, or a tumor cell line. Fibroblasts are particularly useful test cells, but any mammalian cell type can be used because IEGs are ubiquitously expressed. Useful growth factors, cytokines, tumor promoters, and oncogenes include, for example, epidermal growth factor (EGF) and EGF-related factors including, for example, transforming growth factor α (TGFα), heparin-binding-like EGF, heregulin, amphiregulin, epiregulin, cripto, platelet derived growth factor (PDGF), including PGDF-AA, PGDF-BB, and PGDF -CC, insulin, insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), colony stimulating factor (CSF),

and heaptocyte growth factor (HGF). Useful cytokines include, for example, the chemokines, interleukins, and lysophosphatidic acid (LPA). Useful tumor promoters include, for example, phorbol esters, phosphatase inhibitors such as okadaic acid, microcystin, vanadate, hydrogen peroxide. and calyculin A. Useful oncogenes include, for example, Erb2/neu, sis, kit, Ras, Raf, PI3-kinase, and PTEN. Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) are particularly useful growth factors. In other embodiments in which the target protein is c-Fos, the binding of the MAP kinase to c-Fos is assessed by measuring the phosphorylation of T325 or T331. Preferably, this is performed using a phospho-T325-specific antibody.

In another aspect, the invention provides a method for identifying a therapeutic compound by (i) providing a polypeptide that contains a DEF domain, a MAP kinase, and a candidate compound, (ii) mixing the polypeptide, the MAP kinase, and the candidate compound, (iii) measuring the binding of the MAP kinase to the candidate compound, and (iv) identifying the candidate compound as a therapeutic compound, wherein the candidate compound inhibits the binding measured in step (iii), compared to the binding of the MAP kinase to the polypeptide in the absence of the candidate compound. In desirable embodiments, the target protein further contains a fluorescent moiety (e.g., fluorescein).

In preferred embodiments of the previous two aspects, the MAP kinase is ERK1/2. In other desirable embodiments, the target proteins are members of the Fos, Jun, and Myc family proteins. Specifically, desirable target proteins include c-Fos, Fra-1, Fra-2, cMyc, N-Myc, JunD, JunB, c-Jun, in addition to Egr-1 and mPer1. In one embodiment, the target protein contains the sequence of a protein identified in Table 1 or 2 and the identified therapeutic is useful for treating cancer. In another embodiment, the target protein contains the sequence of a protein identified in Table 3 and the identified therapeutic is useful for treating a cardiovascular disease. In another embodiment, the target protein contains the sequence of a protein identified in Table 4 and the identified therapeutic is useful for treating an inflammatory disorder. In another embodiment, the

target protein contains the sequence of a protein identified in Table 5 and the identified therapeutic is useful for treating a metabolic disorder. In another embodiment, the target protein contains the sequence of a protein identified in Table 6 and the identified therapeutic is useful for treating a neuropathy or a behavioral disorder. In another embodiment, the target protein contains the sequence of a protein identified in Table 7 and the identified therapeutic is useful for treating a sleep disorder. In other embodiments, the target protein contains a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1). Assessment of target residue phosphorylation is desirably performed using a phospho-specific antibody.

In another aspect, the invention provides a method for treating cancer in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is a member of the Fos, Jun, and Myc family proteins including, for example, c-Fos, Fra-1, Fra-2, cMyc, N-Myc, JunD, JunB, and c-Jun. Alternatively, the target protein is one identified in Tables 1 or 2. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a cardiovascular disease in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 3. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating an inflammatory disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 4. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a metabolic disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 5. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a neuropathy or a behavioral disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 6. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a sleep disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified

in Table 7. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

Particularly useful DEF domain inhibitors for any of the therapeutic methods are polypeptides having the sequence F/Y—X—F/Y—X (SEQ ID NO: 28; "naked DEF domains") and chimeric proteins that contain a DEF domain inserted into a non-target protein. In preferred embodiments, the DEF domain has the sequence F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1). The most desirable chimeric proteins are based on non-target proteins that affect the pharmacokinetic or pharmacodynamic properties compared to administering the naked DEF domain alone. For example, DEF domains may be incorporated into serum albumin or cereloplasmin.

The compound is administered in an amount, frequency, and duration that is therapeutically effective for treating the diagnosed condition. Desirably, the compound is administered in an amount between 0.01 and 3000 mg/day, more preferably, in an amount between 0.1 and 2000 mg/day, either orally or by injection (i.e., intravenous, intramuscular, or subcutaneous). Alternatively, the compound can be administered as a 0.5% to 25% topical formulation.

Therapy may be provided in any appropriate location: at home, the doctor's office, a clinic, a hospital's outpatient department, or a hospital. Treatment generally begins at a hospital so that the doctor can observe the therapy's effects closely and make any adjustments that are needed. The duration of the therapy depends on the condition being treated, the age and condition of the patient, the stage and type of the patient's disease, and how the patient's body responds to the treatment. Drug administration may be performed at different intervals (e.g., daily, weekly, or monthly).

In another aspect, the invention provides an antibody that specifically binds to phospho-T-325 c-Fos. The antibody may be monoclonal or polyclonal.

In another aspect, the invention provides a pharmaceutical formulation that contains a therapeutic compound identified by either of the first two aspects of the invention, and a pharmaceutically acceptable carrier. The pharmaceutical formulation may be suitable for oral administration, injection, or topical application.

By "specifically binds to phospho-T-325 c-Fos," when referring to the antibodies of this invention, is meant an antibody that binds with high affinity ($<10^{-8}$M) to native c-Fos in which the threonine at amino acid position 325 is phosphorylated, but does not significantly bind to c-Fos in which the T325 is unphosphorylated. Desirably, the difference in specificity of antibody binding between phospho-T-325 c-Fos and the unphosphorylated form is at least 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, or 1000-fold.

By "DEF domain" is meant a polypeptide having the amino acid sequence: F/Y—X_1—F/Y—X_2 (SEQ ID NO: 28), wherein F is phenylalanine, Y is tyrosine, P is proline, and X_1 and X_2 are any naturally-occurring or non-naturally-occurring amino acids. Desirably, X_2 is proline.

By "target protein" is meant any protein that contains a DEF domain capable of binding a target kinase (e.g., a MAP kinase). Desirable target proteins are phosphorylated by the MAP kinase ERK1/2 following ERK1/2 binding to the DEF domain. Target proteins include, for example, gene products of the immediate early genes from the Fos, Myc, and Jun families, proteins identified in Tables 1-7, or chimeric or synthetic proteins into which a DEF domain has bee inserted by artifice. Specific target proteins include, for example, c-Fos, Fra-1, Fra-2, cMyc, N-Myc, JunD, JunB, c-Jun, Egr-1, and mPer1.

By "target residue(s)" is meant one or more residues of a target protein that are N-terminal to the DEF domain and that are phosphorylated as a result of the binding of a MAP kinase. Target residues include, for example, T325 and T331 of c-Fos.

By "primed," when referring to a target protein, is meant a phosphorylation event that makes a DEF domain available for binding of a MAP kinase. Thus, the amino acid residues that are the subject of a "priming" modification are not the same as the target

residues. For example, c-Fos is primed when S362 and/or S374 are phosphorylated or substituted for aspartate or glutamate.

By "target kinase" is meant a protein kinase that is capable of binding a DEF domain and phosphorylating a target residue. Target kinases include the MAP kinases such as ERK1/2, for example. Thus, an "activated target kinase" is one that itself has undergone a post-translational modification causing an increase in kinase activity and/or inducing a change in subcellular localization. For example, in order to be fully activated and translocated from the cytoplasm to the nucleus, ERK1/2 is phosphorylated.

By "MAP kinase" is meant a kinase that recognizes and transfers phosphate to a phosphoacceptor amino acid with the general consensus Ser/Thr-Pro.

By "DEF domain inhibitor" is meant any chemical compound (i.e., polypeptide or non-peptide) that inhibits the interaction of a target kinase (i.e., ERK1/2 or RSK) with the DEF domain of a target protein.

By "assessing," when referring to a step in a method, is meant either qualitative or quantitative assessment of an appropriate endpoint. For example, a qualitative assessment of ERK1/2 phosphorylation is visualization of the ERK localization in the nucleus following an appropriate stimulus. Quantitative assessment may be performed, for example, using Western blotting or enzyme assay.

By "cancer" is meant neoplastic cells multiplying in an abnormal manner. In a cancer, growth is uncontrolled and progressive, and occurs under conditions that would not elicit, or would halt the multiplication of non-cancerous cells. Cancer includes, for example, leukemias and lymphomas (Hodgkin's disease, non-Hodgkin's disease), as well as solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, liposarcoma, osteogenic sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, small and/or non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, schwannoma, meningioma,

melanoma, neuroblastoma, and retinoblastoma.

By "treating cancer" is meant a therapy that measurably slows, stops, or reverses the growth rate of the cancer (i.e., neoplastic cells) *in vivo*. Desirably, a slowing of the growth rate is by at least 20%, 30%, 50%, or even 70%, as determined using a suitable assay for determination of cell growth rates (e.g., a cell growth assay described herein). Typically, a reversal of growth rate is accomplished by initiating or accelerating necrotic or apoptotic mechanisms of cell death in the neoplastic cells, resulting in a shrinkage of the neoplasm. Efficacy of a treatment may be measured by any means known to those skilled in the art including tumor imaging or measurement of neoplastic markers.

By "cardiovascular disease" is meant ischemic heart disease, ventricular heart failure, cardiac hypertrophy, hypertension, and atherosclerosis.

By "inflammatory disorder" is meant any condition that is characterized by inflammation as a primary or secondary symptom. Inflammatory disorders include, for example, allergic or autoimmune disorders, anaphylaxis, and septic shock. Examples of allergic disorders include allergic rhinitis, asthma, atopic dermatitis, and food allergies. Examples of autoimmune disorders include, but are not limited to, type 1 insulin-dependent diabetes mellitus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, dermatitis, meningitis, thrombotic thrombocytopenic purpura, Sjögren's syndrome, encephalitis, uveitis, leukocyte adhesion deficiency, rheumatoid and other forms of immune arthritis, rheumatic fever, Reiter's syndrome, psoriatic arthritis, progressive systemic sclerosis, primary biliary cirrhosis, pemphigus, pemphigoid, necrotizing vasculitis, myasthenia gravis, multiple sclerosis, lupus erythematosus, polymyositis, sarcoidosis, granulomatosis, vasculitis, pernicious anemia, CNS inflammatory disorder, antigen-antibody complex mediated diseases, autoimmune hemolytic anemia, Hashimoto's thyroiditis, Graves disease, habitual spontaneous abortions, Reynard's syndrome, glomerulonephritis, dermatomyositis, chronic active hepatitis, celiac disease, autoimmune complications of AIDS, atrophic gastritis, ankylosing spondylitis and Addison's disease.

By "metabolic disorder" is meant a disease that interferes with the normal metabolic function of cells, tissues or organs. Metabolic disorders include, for example, diabetes, obesity, jaundice, polycystic kidney and hepatic disease, pancreatitis, Graves' disease, and Werner's syndrome. Metabolic diseases may also arise as secondary complications of another disease such as one involving a tumor. For example, cachexia or muscle wasting, and metabolic and digestive complications often arise from the presence of pancreatic, colonic, stomach, hepatic and hepatocellular tumors.

By "neuropathy" is meant any condition of the central or peripheral nervous system characterized by axonal loss that may or may not be accompanied by neuronal loss. Neuropathies specifically include conditions affecting sensory and motor neurons and include, for example, diabetic neuropathy, muscular dystrophy, Williams Beuren's Syndrome.

By "behavioral disorder" is meant any condition affecting motivation, emotion, learning, or memory. Behavioral disorders are also meant to broadly encompass neurodegenerative diseases. Thus, behavioral disorders include, for example, psychosis, schizophrenia, autism, Down's Syndrome, Parkinson's Disease, Alzheimer's Disease, epilepsy, Cockayne syndrome, bipolar disorders, and depression. Also included are addictions including, for example, addictions to opiates and barbiturates.

By "sleep disorder" is meant any condition that primarily affects sleep and consciousness. Sleep disorders include, for example, advanced sleep phase syndrome, delayed sleep phase syndrome, insomnia and narcolepsy.

By "a therapeutically effective amount" is meant the amount of a compound required to treat cancer (i.e., inhibit the growth of the neoplastic cells). The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of neoplasms (i.e., cancer) varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen.

Brief Description of Drawings

FIGURE 1 is a series of photomicrographs showing the differential responsiveness of Swiss 3T3 fibroblasts to growth factors. FIGURE 1A shows quiescent Swiss 3T3 cells (-) that were treated with EGF (25 ng/ml) or PDGF (20 ng/ml) for 20 h and then processed for BrdU incorporation, as described below. FIGURE 1B shows quiescent Swiss 3T3 cells that were treated with PDGF or EGF for the indicated times and ERK1/2 and RSK kinase activities were determined using immunecomplex kinase assays. The fold activation at each time is indicated above each lane. FIGURE 1C is the indirect immunofluorescence detection of hyperphosphorylated activated ERK1/2 in Swiss 3T3 cells treated with EGF or PDGF. FIGURE 1D is the indirect immunofluorescence detection of c-Fos in Swiss 3T3 cells treated with EGF or PDGF.

FIGURE 2A is an illustration showing the residues in c-Fos that are phosphorylated by RSK and ERK1/2 *in vivo*. FIGURE 2B shows the electrophoretic separation of cell extracts from parallel cultures of 208F fibroblasts stably expressing Fos-WT (WT), Fos-AA (AA) or Fos-DD (DD) that were metabolically labelled with ^{35}S-methionine or ^{32}P-orthophosphate and cultured with or without 10% FBS for 15 min. Fos proteins were immunoprecipitated from cell lysates and analysed by SDS-PAGE. The serum-stimulated phosphorylation of Fos-DD was consistently two to threefold greater than Fos-AA and arrows indicate the major mobilities observed after stimulation. FIGURE 2C is Western blot from NIH 3T3 cells transfected with Fos-WT (WT), Fos-AA (AA) or Fos-DD (DD) were serum-starved and then pre-treated with 5 μM UO126 (+) or 0.1% DMSO (-) for 30 min before treatment with EGF (+) for 5 min. FIGURE 2D is an autoradiogram of an *in vitro* phosphorylation of the indicated $(His)_6$-Fos proteins by endogenous ERK1/2 from quiescent or EGF-stimulated NIH 3T3 cells. Results shown are representative of three independent experiments. Fos-EE (S362E/S374E) was used as primed c-Fos for *in vitro* phosphorylation studies.

FIGURE 3A is an illustration that details the DEF domain at the C-terminus of c-Fos. The phosphorylation of Fos-EE in the absence of peptide competitor is expressed

as 100%. *In vitro* phosphorylation of $(His)_6$-Fos-EE was performed as described below. The data shown are the means ±SEM from three experiments. FIGURE 3B is a graph showing the inhibition of Fos-EE phosphorylation by peptides containing a DEF domain (FQFP; SEQ ID NO: 3) or a mutated DEF domain (AQAP; SEQ ID NO: 4). FIGURE 3C is a graph showing the inhibition of Fos-EE phosphorylation by peptides containing the c-Fos DEF domain (FTYP; SEQ ID NO: 2) or a mutated DEF (ATYP; SEQ ID NO: 5). FIGURE 3D shows the results of a Western blot of NIH 3T3 cells transfected with the indicated FLAG-Fos-DD (DD) alleles were left quiescent (-) or were stimulated (+) with EGF for 5 min before lysis. Arrows indicate the major Fos-DD mobilities. FIGURE 3E is a Western blot of cells transfected with the indicated FLAG-Fos alleles. Arrows show the three major Fos-WT mobilities associated with growth factor stimulation.

FIGURE 4A is an illustration identifying the ERK1/2 phosphorylation sites N-terminal to the DEF domain in c-Fos. *In vitro* phosphorylation of $(His)_6$-Fos-EE proteins by activated $(His)_6$-ERK1/2 was performed. The phosphorylation of the Fos-EE point mutants is expressed as a percentage of Fos-EE (100%). The data shown are the means ±SEM from three experiments. FIGURE 4B is a Western blot of NIH 3T3 cells that were transfected with the indicated FLAG-Fos-DD (DD) alleles. Cells were treated with EGF for 5 min or left untreated. FIGURE 4C is a Western blot of cells transfected with the indicated FLAG-Fos alleles and treated as in Figure 3B.

FIGURE 5A is an illustration identifying the phospho-Thr 325 peptide used to generate the phospho-Thr-325-specific anti-c-Fos antiserum. FIGURE 5B is a Western blot of NIH 3T3 cells that were transfected with the indicated c-Fos alleles or with vector alone. Extracts were prepared from quiescent (-) or EGF-stimulated (+) cells and analyzed using either the anti-c-Fos antibody or the phospho-Thr 325 antiserum. Results shown are representative of three independent experiments. FIGURE 5C is a Western blot of ΔB-Raf-ER NIH 3T3 cells transfected with Fos-WT or Fos-AA that were either starved and left untreated (0) or treated with 1 μM tamoxifen (TAMX) for the indicated times before lysis. The *in vivo* phosphorylation of Thr 325 in Fos-WT and Fos-AA was

analyzed by western blotting using the phospho-Thr 325-specifi antiserum. FIGURES 5D and 5E are Western blots demonstrating the *in vivo* mitogen-regulated phosphorylation of Thr 325 in the context of the Fos DEF domain mutants. NIH 3T3 cells expressing the indicated Fos proteins were treated as in the same manner as for FIGURE 3B, and extracts analyzed for phosphorylation of Thr 325.

FIGURE 6A is a Western blot of quiescent Swiss 3T3 cells were treated with EGF (25 ng/ml) for the indicated times. Lysates were probed for endogenous c-Fos, Thr 325 phosphorylation in c-Fos. FIGURE 6B is a Western blot of Swiss 3T3 cells that were treated with PDGF-BB (20 ng/ml) and processed as in described for Figure 6A. Results shown are representative of three experiments. FIGURE 6C is a Western blot of quiescent Swiss 3T3 cells that were treated with PDGF for 60 min and then treated with UO126 (5 μM), as indicated, or with DMSO (lanes 3-8) for the remainder of the experiment. The expression and phosphorylation of endogenous c-Fos was visualized as in above. FIGURE 6D is an autoradiogram showing the kinase activities of endogenous ERK1/2 and RSK in cell lysates from Figure 6C. The fold activity is provided above each lane.

FIGURE 7A is a bar graph showing the AP-1 transcriptional activity of the indicated c-Fos alleles in Hela cells. AP-1 luciferase activity in cells expressing Fos-WT is expressed as 100%, and the data shown are from five individual experiments. FIGURE 7B is a photomicrograph showing the expression of endogenous c-Fos in quiescent (-) or serum-stimulated (+) pMV7-infected 208F fibroblasts (vector), assayed by immunofluorescence microscopy. To examine the expression of Fos-WT, Fos^{Y45A} or $Fos^{T325A/T331A}$ in G418-resistant 208F fibroblasts, cells were serum-starved for 24 h before fixation and processing for immunofluorescence microscopy using the anti-Fos antibody.

FIGURE 7C is a Western blot of c-Fos protein expression in quiescent 208F cells.

FIGURE 7D is a bar graph showing the anchorage-independent growth of G418-resistant pools of 208F cells stably expressing pMV7 (vector) or the indicated FLAG-Fos alleles. The data are expressed as a percentage of the number of colonies formed by cells

expressing Fos-WT (100%) and represent the mean ±SEM from six experiments performed in duplicate.

FIGURE 8 is a schematic diagram of the molecular interpretation of ERK1/2 signal duration. Growth factor stimulation (stimulus) causes activation of signaling pathways (signals) that result in rapid transcriptional induction of immediate early genes (response). The duration of ERK1/2 signaling is then interpreted by immediate early gene products that contain DEF domains (signal sensors). ERK1/2-docking to the DEF domain results in sensor phosphorylation. Docking and phosphorylation alters its biological activity, and this dictates the biological outcome. TF, transcription factor.

FIGURE 9A is a photomicrograph showing the nuclear accumulation of active ERK1/2 and c-Fos in growth factor-treated Swiss 3T3 cells. These photomicrographs are enlargements of the images of Figure 1C in order to visualize the cellular distribution of activated ERK1/2 and nucleolar structures. FIGURE 9B is a photomicrograph of quiescent Swiss 3T3 cells treated with PDGF for 75 minutes followed by the addition of cyclohexamide (+) or vehicle (-). Cells were processed for c-Fos immunofluorescence 90, 180 or 300 minutes after PDGF stimulation. In control experiments (bottom two panels), cells were incubated with cyclohexamide or vehicle for 20 minutes prior to treatment with PDGF for 90 minutes.

FIGURE 10A is a Western blot of NIH3T3 cells transiently transfected with FosWT or FosDD were left quiescent or treated with EGF (50 ng/ml, 5 min) prior to lysis. An aliquot from each cell extract was incubated in the presence or absence of λ protein phosphatase (P'ase) for 30 minutes on ice. Data shown is representative of three separate experiments. FIGURE 10B is a Western blot of NIH3T3 cells stably expressing ΔB-Raf:ER that were transfected with FosWT, FosAA or FosDD. Cells were deprived of serum growth factors, pre-treated with 5 μM UO126 (+) or 0.1% DMSO (-) for 30 minutes prior to treating with tamoxifen (TAMX, 1μM) for 15 minutes prior to cell lysis. FIGURE 10C is a Western blot showing the phosphorylation of $(His)_6$-FosWT, AA or EE or MBP by Flag-ERK5. Active (+) and inactive ERK5 (-) was obtained by

coexpressing Flag-ERK5 and HA-MEK5(D) or control vector, respectively, in 293 cells followed by immunoprecipitation of Flag-ERK5 from cell lysates using the M2 anti-Flag monoclonal antibody. Autophosphorylation (auto-P) of ERK5 in kinase reactions is indicated. Together, these data demonstrate that the phosphorylation of primed c-Fos is regulated by the Raf/Mek/ERK pathway.

FIGURE 11 is a Western blot of quiescent Rat-1 cells that were treated with the indicated concentrations of LPA for various times. The activation kinetics of ERK1/2 demonstrates that c-Fos is a sensor for sustained ERK1/2 signaling in Rat-1 fibroblasts. The data shown is representative of at least three individual experiments.

FIGURE 12 is a series of cell culture plates, fixed and then stained with Giemsa to visualize foci. The indicated Fos proteins were stably expressed in 208F cells and cultured for four weeks in regular culture medium. Identical data was obtained from five separate experiments. Thus, substituting aspartic acid for T235 and T331 in c-Fos promotes Fos-mediated transformation.

FIGURES 13A-C are a series of Western blots showing the regulation of ectopically expressed Fos family proteins (c-Fos, Fra-1, and Fra-2) by the ERK1/2 pathway in NIH 3T3 cells. Cells were treated with or without EGF (50 ng/mL) for 5 minutes prior to cell lysis. Where indicated, UO126 (5 mM) was added to cells 30 minutes before adding EGF. EGF treatment in the absence of UO126 activated ERK1/2, as demonstrated by the mobility shift to a higher molecular weight. ERK1/2 activation resulted in phosphorylation of the target amino acid, T325, of c-Fos (Figure 13A). ERK1/2 activation of Fra-1 (Figure 13B) and Fra-2 (Figure 13C) is also demonstrated by the observed mobility shift.

FIGURE 14A is a sequence alignment of c-Fos, Fra-1, and Fra-2 (SEQ ID NO: 25-27, respectively) demonstrating a high degree of sequence identity in the C-termini. Fra-1 and Fra-2 have ERK1/2 and RSK priming phosphorylation sites in addition to DEF domains. FIGURE 14B is a Western blot of NIH 3T3 cells transfected with the indicated constructs and deprived of serum growth factors for 24 hours. These results demonstrate

that mutations in the DEF domains of Fra-1 and Fra-2 inhibit the ERK1/2-mediated mobility shift (compare to Figures 13B and 13C).

FIGURE 15A is a Western blot of c-Myc immunoprecipitation from NIH 3T3 cells transfected with pcDNA3 (vector) or c-Myc and deprived of serum growth factors. EGF and UO126 were used to treat cells as described in Figure 13. FIGURE 15B is a Western blot from cells transfected with the indicated c-Myc proteins. These results characterize the DEF domain in c-Myc and show that S62 phosphorylation depends on an intact DEF domain.

FIGURES 16A-F are Western blots demonstrating the kinetics of immediate early gene expression in Swiss 3T3 cells. Cells were deprived of serum growth factors and treated with EGF (25 ng/mL) or PDGF-BB (20 ng/mL) for the indicated times.

FIGURES 17A-B are Western blots demonstrating the kinetics of Egr-1, JunB, and c-Myc expression in Swiss 3T3 cells. Cells were treated as described in Figure 13. Total levels of c-Myc (Figure 17B) were detected by immunoprecipitating c-Myc prior to Western analysis.

FIGURES 18A-E are Western blots demonstrating that sustained expression of immediate early genes requires ERK1/2 activity. Serum deprived Swiss 3T3 cells were treated with PDGF-BB for 90 minutes before adding DMSO vehicle (0.1%) or UO126 (5 μM).

FIGURE 19A is a Western blot of cells treated with PDGF-BB for either 90 minutes (lanes 2-9) or 120 minutes (lanes 11-13) before adding DMSO vehicle (lanes 3-5) or UO126 (lanes 7-9, 12, 13). FIGURE 19B is a Western blot of cells treated with PDGF-BB for 5 hours to induce Fra-1 before adding UO126 for a further 20 or 30 minutes. These figures demonstrate that ERK1/2 signaling is required during G1 for the stabilization of c-Myc.

FIGURE 20 is a series of Western blots from Swiss 3T3 cells treated with various concentrations of PDGF-BB before lysis. These results demonstrate that IEG products act as sensors for subtle differences in ERK1/2 signal duration.

FIGURE 21A is a bar graph of the result from an *in vitro* kinase assay demonstrating ERK 1/2 activation is sensitive to small differences in growth factor (PDGF) stimulation. FIGURE 21B is a Western blot demonstrating that the c-Fos stabilization observed in Figure 20 following stimulation with 10 ng/ml PDGF is a result of ERK 1/2-dependent phosphorylation of T325. Neither long-term c-Fos stabilization (see Figure 20) nor T325 phosphorylation is observed following 4 ng/ml PDGF stimulation.

FIGURE 22 are Western blots showing Fra-1 hyperphosphorylation throughout G1 requires ERK1/2 signaling.

FIGURE 23 is representative gel and the densitometric quantification of an electrophoretic mobility shift assay (EMSA) for AP-1. Swiss 3T3 cells were treated as indicated and extracted in a hypotonic lysis buffer. The nuclear fraction was isolated and aliquots mixed with a ^{32}P-labelled AP-1 oligonucleotide in a standard EMSA. These results demonstrate that PDGF, but not EGF, treatment significantly increases AP-1 expression and AP-1 DNA binding.

FIGURE 24 is an immunoprecipitation of extracts from 208F cells stably expressing c-Myc or c-Myc F196A and treated with cycloheximide (14 mg/mL) for the indicated times. Following immunoprecipitation of the c-Myc proteins, total levels of c-Myc were detected using Western analysis. These results demonstrate that c-Myc stability requires the DEF domain.

FIGURE 25 is a series of indirect immunofluorescence photomicrographs demonstrating typical results of the screening assays described herein. Representative fields of view using a 10X objective lens are shown.

Detailed Description

We have discovered that DEF domains are present in numerous proteins that are important in a variety of human diseases and, by blocking the interaction of a MAP kinase with the DEF domain of a target protein, effective therapy may be provided. Also

provided are screening methods for identifying novel therapeutics that inhibit the MAP kinase-DEF domain interaction. This invention provides several advantages over known therapies that directly target the MAP kinase signaling cascade. Typically, most compounds that inhibit the MAP kinase pathway are non-specific and inhibit more than one enzyme. Also, the targeted kinases, if effectively inhibited, are not available to perform normal physiological functions necessary for cell survival, resulting in toxicity to healthy as well as diseased cells. By contrast, the therapeutic methods of the present invention inhibit the activation of particular target proteins, leaving the MAP kinases enzymatically active and available to phosphorylate other, non-DEF domain-containing proteins. Diseased cells (e.g., cancerous cells) are often more susceptible to therapy because of the higher concentration of target protein.

The principles of the invention are exemplified using the immediate early gene, c-Fos, but is not intended to be limiting. c-Fos functions as a molecular sensor for the duration of extracellular-signal-regulated kinase 1/2 (ERK1/2) signaling. c-Fos is known to be phosphorylated by ERK1/2 and RSK, resulting in increased stability of the protein. Therefore, the biological function of c-Fos differs under conditions where ERK1/2 signaling is sustained, rather than transient. Signaling is transduced by ERK1/2 binding to the DEF domain of c-Fos. Mutating the DEF domain inhibits c-Fos-mediated signaling and, ultimately, the downstream effects of ERK1/2 activation.

Further, Fos, Myc and Jun family proteins are transcription factors encoded by immediate early protooncogenes. Family members c-Fos, Fra-1, Fra-2, c-Myc, N-Myc, JunD, and JunB are frequently found to be amplified or upregulated in human cancers. Sustained ERK1/2 signaling is required for cell proliferation and ERK1/2 docking to these proteins occurs only when signaling is sustained. Docking controls the growth-promoting function of these transcription factors. Accordingly, ERK1/2 docking inhibitors may be clinically useful drugs because they will likely to inhibit a specific branch of ERK1/2 signaling and would, therefore, be less toxic than general ERK1/2 inhibitors.

Sustained ERK1/2 Activation Correlates With S Phase Entry

Treatment of quiescent Swiss 3T3 fibroblasts with platelet-derived growth factor (PDGF) stimulated S phase entry; whereas, treatment with epidermal growth factor (EGF) did not (Figure 1A). The activation kinetics and amplitude of ERK1/2 and RSK, however, were almost identical following a 5-10 minute exposure to either PDGF or EGF (Figure 1B). In both cases, hyperphosphorylated (active) ERK1/2 was localized to the nucleus (Figure 1C). In contrast to ERK1/2 and RSK activation kinetics, rates of inactivation were faster in cells treated with EGF compared to PDGF (Figure 1B). ERK1/2 signaling remained elevated for at least 240 minutes following PDGF exposure, but returned to basal levels within 30-45 minutes following EGF withdrawal (Figure 1B). Notably, the sustained ERK1/2 activity elicited by PDGF treatment remained localized to the nucleus (Figures 1C and 9A), demonstrating a tight correlation with S phase entry. Further, c-Fos protein expression was prolonged in cells treated with PDGF compared to those treated with EGF (Figure 1D). This indicates that c-Fos becomes stabilized when ERK1/2 signaling is prolonged, but is unstable when ERK1/2 signaling is transient. c-Fos expression was not affected by either the addition of cycloheximide to cells 75 minutes after PDGF treatment (Figure 9B) or the addition of actinomycin D 20 minutes after PDGF treatment. Thus, the differences in c-Fos expression between PDGF- and EGF-treated cells arises from post-translational control. This conclusion is further supported by studies showing that the transcriptional induction of *c-fos* and other IEGs by various growth factors is completed within 30-45 minutes.

ERK1/2 and RSK coordinately phosphorylate the extreme C-terminus of c-Fos at Ser 374 and Ser 362, respectively (Figure 2A). Mutating these residues to aspartate (Fos-DD), which mimics phosphorylation, results in enhanced transformation of fibroblasts by comparison to c-Fos having Ser 362 and Ser 374 mutated to alanine (Fos-AA) (Okazaki *et al., EMBO J.*, 14: 5048-5059, 1995; Chen *et al., Proc. Natl. Acad. Sci. USA*, 90: 10952-10956, 1993). Thus, increasing the stability of c-Fos is not the only manner in which this

transcription factor can regulate cellular transformation. Fos-AA appears to be differentially regulated compared to Fos-DD.

We have discovered that the addition of serum to fibroblasts results in a large, λ phosphatase-sensitive electrophoretic mobility shift of Fos-DD, compared to Fos-AA (Figures 2B left, and 10A). This effect correlates with increased incorporation of ^{32}P-orthophosphate that was consistently two to threefold greater for Fos-DD than Fos-AA (Figure 2B, right). This demonstrates that phosphorylation of Ser 362 and Ser 374 prime c-Fos for additional growth factor-regulated phosphorylation. As Fos-DD has greater transforming potential than Fos-AA, the regulation of primed c-Fos is critical for promoting fibroblast proliferation.

Phosphorylation of Primed c-Fos is MEK-dependent

NIH 3T3 cells transfected with different Fos proteins were treated with the MEK inhibitor UO126 (Favata *et al., J. Biol. Chem.*, 273: 18623-18632, 1998) to determine if the mitogen-regulated phosphorylation of Fos-DD is mediated by the Raf/MEK/MAPK pathway. UO126 inhibited the growth factor-regulated mobility shift of Fos-WT and Fos-DD (Figure 2C) and ERK1/2 activation (Figure 2C, bottom), indicated that ERK1/2 or downstream signaling molecules regulated primed c-Fos. Identical observations were made using NIH 3T3 cells expressing a conditionally active form of B-Raf (ΔB-Raf-ER) and treating these cells with tamoxifen instead of EGF (Figure 10B). To determine whether ERK1/2 could phosphorylate primed c-Fos *in vitro*, we used different hexahistidine-Fos fusion proteins as substrates. ERK1/2 efficiently phosphorylated Fos-WT (Figure 2D). The phosphorylation of Fos-AA and primed c-Fos, Fos-EE (S362E/S374E), by ERK1/2 was also easily detected *in vitro*, but the phosphorylation of Fos-EE compared with Fos-AA was consistently greater (Figure 2d). These results demonstrate that ERK1/2 can phosphorylate sites in c-Fos other than Ser 374 and that this phosphorylation is enhanced after priming of the C terminus. In contrast to ERK1/2, phosphorylation of c-Fos by ERK5 *in vitro*, another UO126-sensitive proline-directed

kinase, was not observed (Figure 10C).

An ERK1/2 Targeting Motif Promotes the Phosphorylation of Primed Fos

The preference of ERK1/2 for primed c-Fos (Fos-EE) over Fos-AA demonstrates that C-terminal phosphorylation exposes additional phosphorylation sites and/or a motif that would increase the efficiency of phosphorylation at these sites. Examination of the c-Fos sequence identified a site in the C terminus that has similarity with the ERK1/2 targeting motif, FXFP (SEQ ID NO: 1), known as a DEF domain. In c-Fos, this motif is FTYP (Figure 3A; SEQ ID NO: 2). Mutating either Phe 343 or Tyr 345 to alanine dramatically inhibited the phosphorylation of primed c-Fos (Fos-EE) by ERK1/2 *in vitro* (Figure 3A). ERK1/2-regulated phosphorylation of substrates that contain DEF domains can be competitively inhibited *in vitro* with a synthetic peptide encompassing the DEF domain found in ELK-1. This peptide (FQFP; SEQ ID NO: 3) inhibited the phosphorylation of primed c-Fos in a concentration-dependent manner (Figure 3B). By contrast, a peptide with a mutant DEF domain (AQAP; SEQ ID NO: 4) was less efficient in inhibiting ERK1/2-mediated phosphorylation of primed c-Fos. The ELK-1 peptide was then engineered to contain the c-Fos FTYP DEF domain (SEQ ID NO: 2). This peptide also inhibited primed c-Fos phosphorylation, but a mutant version (ATYP; SEQ ID NO: 5) did not (Figure 3C). In both cases, the IC_{50} for the FQFP (SEQ ID NO: 3) and FTYP (SEQ ID NO: 2)peptides was approximately 80 μM. The EGF-stimulated mobility shift of Fos-DD *in vivo* was also inhibited when Phe 343 or Tyr 345 were mutated to alanine (Figure 3D). These results demonstrate that the initial phosphorylation of c-Fos by ERK1/2 and RSK as the extreme C terminus expose an ERK1/2 docking site that allows ERK1/2 to phosphorylate additional sites.

Based on this model, mutation of Phe 343 or Tyr 345 to alanine should prevent hyperphosphorylation of Fos-WT and not interfere with the priming phosphorylations, which are C-terminal to the DEF domain. Indeed, these mutations prevented the appearance of the slowest mobility, but still allowed a shift to the intermediate mobility

(Figure 3E). This indicates that the DEF domain is not involved in directing ERK1/2 to prime c-Fos through Ser 374 phosphorylation. Instead, ERK1/2 docking through the DEF domain results in the hyperphosphorylation of primed c-Fos.

ERK1/2 phosphorylates Thr 325 and Thr 331 in primed c-Fos

There are two proline-directed threonine residues (Thr 325 and Thr 331) amino-terminal to the DEF domain in c-Fos (Figure 4A). Mutation of Thr 325 to alanine almost completely inhibited the phosphorylation of Fos-EE by ERK1/2 *in vitro*, and the additional mutation of Thr 331 to alanine was required to reduce the phosphorylation to background levels (Figure 4A). Thr 325 and Thr 331 were also phosphorylated in primed c-Fos (Fos-DD) *in* vivo, as evidenced by the complete loss of the mobility shift in the T325A/T331A mutant (Figure 4B). Individual mutation of Thr 325 or Thr 331 to alanine in the context of Fos-DD only partially inhibited growth factor-regulated phosphorylation. Substituting alanines for Thr 325 and Thr 331 in the context of Fos-WT prevented the EGF-stimulated to the slowest mobility (Figure 4C). However, EGF treatment was associated with the appearance of the intermediate mobility form, resulting from priming phosphorylation of ERK1/2 and RSK. Collectively, these observation demonstrate an ordered phosphorylation process whereby the initial phosphorylation of c-Fos at Ser 374 and Ser 362 (priming) exposes a DEF domain that mediates the hyperphosphorylation of c-Fos at Thr 325 and Thr 331. Further, Ser 374 phosphorylation is not regulated by docking. This is consistent with the phosphorylation of amino acids N-terminal, but not C-terminal, to DEF domains.

A phosphorylation-specific antiserum for Thr 325 in c-Fos (Figure 5A) was generated to investigate the mitogen-regulated phosphorylation of this residue in primed c-Fos. The antiserum showed little or no reactivity with Fos-WT or Fos-DD expressed in quiescent cells (Figure 5B, -EGF). However, after treatment with EGF, strong reactivity was associated with Fos-WT and Fos-DD, but not with Fos^{T325A} or $Fos\text{-}DD^{T325A}$ (Figure 5B, +EGF). Priming of the extreme C terminus by ERK1/2 and RSK promotes additional

phosphorylation of c-Fos *in vivo* (Figure 2B). To determine if this is caused by increased phosphorylation of Thr 325, Fos-WT and Fos-AA were expressed to similar levels in the ΔB-Raf-ER NIH 3T3 cells that were then treated with tamoxifen for varying times (Figure 5C). The phosphorylation of Thr 325 was greater in cells transfected with Fos-WT than those transfected with Fos-AA.

Mutating Phe 343 or Tyr 345 to alanine prevented the hyperphosphorylation of primed c-Fos (Figure 3). Specifically, the regulation of Thr 325 phosphorylation *in vivo* was inhibited when Phe 343 or Tyr 345 were mutated to alanine, either in the context of Fos-WT (Figure 5D) or Fos-DD (Figure 5E). In this later experiment, there is a strong correlation between the mobility shift of Fos-DD and increased Thr 325 phosphorylation confirming that the DEF domain in c-Fos increases the efficiency of Thr 325 phosphorylation *in vivo*.

The phosphorylation of Thr 325 is differentially regulated by ERK1/2-signal duration

As shown above, the induction kinetics of c-Fos expression 30-45 min after addition of PDGF or EGF to Swiss 3T3 cells were identical (Figure 1D). This is consistent with a model in which an initial activation of ERK1/2 or RSK is sufficient for induction of *c-fos* IEG expression. To determine if ERK1/2 signal duration differentially regulates the phosphorylation of Thr 325 in endogenous c-Fos, we prepared extracts from Swiss 3T3 cells treated with EGF or PDGF for different times. Importantly, although c-Fos is present in cells after 45 or 60 min of EGF treatment, Thr 325 phosphorylation was not observed (Figure 6A). This is consistent with inactivation of ERK1/2 occurring before c-Fos is present (Figure 6A). By contrast, phosphorylation of Thr 325 increased 45-60 min after addition of PDGF (Figure 6B). Maximal Thr 325 phosphorylation persisted for at least 120 min (Figure 6B), but was still detected after 240 min. In Rat-1 fibroblasts, treatment with 100 μM lysophosphatidic acid (LPA) results in sustained ERK1/2 activity and S phase entry, whereas treatment with 0.1-1 μM LPA transiently

activates ERK1/2 and no cell cycle progression occurrs. Although treatment of quiescent Rat-1 fibroblasts with mitogenic (100 μM) and no-mitogenic (0.5 μM) concentrations of LPA resulted in a similar induction of *c-fos*, phosphorylation of Thr 325 only occurred with 100 μM LPA (Figure 11). These findings correlate with the generation of transient and sustained ERK1/2 responses by 0.5 μM and 100 μM LPA, respectively (Figure 11). Thus, differential phosphorylation of c-Fos occurs in different cell types and in response to agonists that directly activate tyrosine kinase receptors or heterotrimeric G protein-coupled receptors.

To show that the sustained phase of ERK1/2 signaling was required to mediate the stabilization and hyperphosphorylation of endogenous c-Fos in Swiss 3T3 cells, ERK1/2 and RSK activity was inhibited by adding UO126 to cells that had been treated with PDGF for 60 min (Figure 6D). Under this condition, the phosphorylation of Thr 325 was completely inhibited and the electrophoretic mobility of c-Fos increased (Figure 6C). Importantly, these UO126-induced changes in the biochemical properties of c-Fos also preceded the rapid disappearance of c-Fos protein. This result is consistent with hypophosphorylated c-Fos being unstable. These observation show that the phosphorylation of c-Fos at Thr 325 is tightly correlated with the activation/inactivation kinetics of ERK1/2 in different cell types of provide clear evidence that c-Fos can function as sensor for ERK1/2 signal duration.

The DEF domain and Thr 325/Thr 331 phosphorylation modulates c-Fos function

In Hela cells expressing Fos-WT, AP-1 transcription factor activity was consistently three to fourfold above background levels (Figure 7A). Mutation of Thr 325 and Thr 331 to alanine reduced AP-1 activity by about 20%; whereas, mutating the DEF domain (F343A) reduced Fos-WT activity by about 65% (Figure 7A). These observations indicate that docking of ERK1/2 to c-Fos is important in regulating c-Fos transcriptional activity under conditions of growth factor stimulation. To determine if ERK1/2 docking to c-Fos can contribute to c-Fos function independently of the

phosphorylation-mediated stabilization, Fos-WT, $Fos^{T325A/T331A}$ or Fos^{Y345A} (a DEF domain mutant) were stably expressed in 208F fibroblasts. The expression of the different Fos proteins (Figure 7B, bottom six panels) was equivalent to the level of endogenous c-Fos in serum-stimulated vector-infected cells (Figure 7B, top two panels) and was also localized to the nucleus. Western analysis of c-Fos expression in the quiescent cell lines also showed that they were expressed to similar levels (Figure 7C). The stable expression of Fos-WT promoted anchorage-independent growth in soft agar (Figure 7D), as expected. Mutating Thr 325 and Thr 331 to alanine significantly reduced the growth of 208F cells in soft agar suggesting that phosphorylation of these residues promotes cellular transformation (Figure 7D). However, replacing Thr 325 and Thr 331 with Asp enhanced c-Fos-mediated focus formation (Figure 12). Further, mutating the c-Fos DEF domain (Fos^{Y345A}) completely inhibited the ability of c-Fos to transform 208F cells; more so than the c-$Fos^{T325A/T331A}$ mutant (Figure 7D). These results demonstrate that ERK1/2 docking to c-Fos contributes to transformation through mechanisms in addition to Thr 325/Thr 331 phosphorylation and that stabilization of c-Fos is not the only factor that regulates c-Fos function, as all proteins were expressed equally. In addition, it also demonstrates that ERK1/2 docking to c-Fos regulates biological activity.

Mechanism of IEG Activation Through DEF Domain Binding

The mechanism described here employs an IEG product, typified by c-Fos, which functions as a molecular sensor that differentiates between differences in ERK1/2 and RSK signal duration, as well as their cytoplasmic/nuclear distribution (Figure 8). As observed for a large number of IEGs, the *c-fos* gene is transcriptionally induced within minutes of growth factor stimulation and therefore occurs with kinetics that are independent of differences in signal duration. Newly synthesized c-Fos protein has a half-life of about 30-45 min but, when phosphorylated by ERK1/2 and RSK, the half-life is extended to at least 2h. Thus, when ERK1/2 is rapidly activated (transient signal), c-Fos is present in the nucleus, but is not phosphorylated, and is therefore unstable and

degraded (Figure 8). By contrast, delayed inactivation of ERK1/2 (sustained signal) results in the efficient phosphorylation of c-Fos at its extreme C terminus, resulting in its stabilization for several hours. The initial priming phosphorylation in the C-terminus exposes a DEF domain that promotes additional ERK1/2-mediated phosphorylation events, increasing the efficiency of ERK1/2-regulated phosphorylation when ERK1/2 is only sub-maximally active (0.5-4 h after stimulation). Further, when priming and docking are inhibited by point mutation (Fos-AA and Fos^{F343A} or Fos^{Y345A}, respectively) ERK1/2 and/or RSK signals are unable to alter c-Fos function. These non-phosphorylable c-Fos mutants likely resemble the hypophosphorylated form of c-Fos that is present when ERK1/2 is rapidly inactivated during transient signaling and cells do not enter S phase.

Simply prolonging the half-life of c-Fos will not affect its role in promoting transformation. Instead, the combination of protein stabilization and DEF-mediated regulation allows c-Fos to function as sensor for ERK1/2. If c-Fos is not stabilized during the sustained phase of signaling, ERK1/2 will not target the c-Fos DEF domain. Therefore, stabilizing the IEG product is a critical first step if it is to function as a sensor for sustained ERK1/2 signals. The physiological importance of the c-Fos DEF domain is underscored by the fact that mutations in the DEF domain significantly reduced AP-1 activity and inhibit the transforming activity associated with wild-type c-Fos. However, the effect of mutating the DEF domain is stronger than the effect of mutating the phosphorylation sites that are controlled by this docking site, indicating that the DEF domain can have more than one action. An additional function of the DEF domain ERK1/2-mediated *trans*-phosphorylation of AP-1 complex proteins.

We described a general mechanism for cellular sensing of ERK1/2 signal strength and timing involving the FTYP (SEQ ID NO: 2) DEF domain present in many IEGs. Putative DEF domains are found in additional AP-1 proteins, such as Fra-1, Fra-2, Jun-B and JunD (Table 1). The proto-oncogene products c-Myc and N-Myc also contain putative DEF domains. The IEG product Egr-1 has a DEF domain and several putative proline-directed phosphorylation sites N-terminal to this domain that could enable Egr-1

to sense sustained signaling in PC12 cells and promote neuronal differentiation. In common with the c-Fos DEF domain, the other DEF'domains highlighted in Table 1 show subtle deviation form the FXFP consensus (SEQ ID NO: 1), with respect to the presence of phenylalanine at positions 1 and 3 indicating that tyrosine can be tolerated at either site.

Table 1. DEF Domains in Immediate Early Gene Products

IEG	*Amino Acid Sequence*	*SEQ ID NO.*
c-Fos	^{314}-GPMVTELEPLCTP-VVTCTPSCTTYTSSFV**FTYP**EEADS	6
Fra-1	^{211}-GP-VLEPEALHTPTLMT-TPSLTPFTPSLV**FTYP**STPEP	7
Fra-2	^{169}-GGFYGE-EPLHTP-IVVTSTPAITPGTSNLV**FTYP**SVLEQ	8
Fos-B	^{283}-HSEVQV-LGDPFPVV-SPS-YTSSFVLTCPEVSAF	9
JunD	^{87}-LLASPDLGLLKLASPELERLIIQS-NGLVTTTPTST-Q**FLYP**KV	10
JunB	^{71}-GQGSDTGASLKLASSELERLIVPNSNGVITTTPTPPGQ**YFYP**RG	11
c-Jun	^{60}-LLTSPDVGLLKLASPELERLIIQSSNGHITTTPTPT-QFLCPKN	12
c-Myc	^{56}-LPTPPLSPSRRSGLCSPSYV ^{181}-LTA-AASECIDPSVV**FPYP**LND	13 14
N-Myc	^{77}-AQSPGAGAASPAGRGHGGAAGA ^{110}-AHPAAECVDPAVV**FPFP**VNK	15 16
Egr-1	^{184}-QSPPLSCAVPSNDSSPIYSAAPTFPTPNTD ^{247}-PMIPD**YLFP**QQ	17 18
MPer1	^{697}-PRGGPQPLPPAPTSVPPAAFPAPLVTPMVALPN**YLFP**TPSSY	19

DEF domains are in bold and number indicate amino acid position. Sequences are from rat (c-Fos, Fra-1, and Fra-2), mouse (FosB, JunD, c-Jun, c-Myc, and Egr-1), or human (JunB, N-Myc, and mPer1).

Screening Methods to Identify Inhibitors of DEF Domain Binding

DEF Domain Binding Assessment using a Phospho-specific Antibody

We have developed a cell-based assay which is used to screen small molecule compound libraries (Figure 25). In this assay, rat 208F fibroblasts that stably express c-Fos are cultured in a 384-well plate and deprived of serum growth factors for 24 hours. Cells are then treated with EGF for 15 minutes and fixed with 3.7% formaldehyde. Permeabilized fixed cells are incubated with DAPI to stain the nuclei and an anti-phospho-ERK1/2 mouse monoclonal antibody and an anti-phospho-T325 Fos rabbit polyclonal antibody for 2 hours. Anti-mouse Alexa594-conjugated IgG and an anti-rabbit FITC-conjugated IgG are added to each well and unbound antibody is removed by several washes. The fluorescence intensity of both fluorophores in each well can be detected using an automated epifluorescence microscope or Autoscope (Universal Imaging Systems, Inc.). A clear increase in T325 phosphorylation was observed in cells treated with EGF (indicating that ERK1/2 docking to Fos has taken place). In the same population of cells, the phosphorylation of ERK1/2 also increased, thus indicating its activation by EGF. Under these conditions, only background levels of fluorescence were detected when both phospho-specific antibodies were omitted, and the secondary antibodies show no cross-species reactivity.

Inhibition of ERK1/2 docking to the c-Fos DEF domain in vivo could result from compounds that (a) are generally toxic, (b) prevent the activation of ERK1/2, (c) prevent the growth factor regulated translocation of ERK1/2 into the nucleus where c-Fos is localized or (d) directly antagonize ERK1/2 docking to the DEF domain. The assay we have developed naturally excludes the first three possibilities. First, toxicity will be reflected by nuclear integrity as visualized with DAPI staining. Second, inhibition of ERK1/2 activation/activity will be apparent from the phospho-ERK1/2 fluorescence signal. Third, nuclear translocation of ERK1/2 can be verified by manually examining images from wells that show decreased c-Fos phosphorylation. Therefore, candidate compounds that specifically inhibit ERK1/2 binding to the DEF domain are defined as

compounds that decrease the phosphorylation of T325 in c-Fos but which have no effect on ERK1/2 activation or its localization. Although this assay is exemplified using rat fibroblasts, it may be performed using any appropriate cell type including, for example, myoblasts, epithelial cells, and hepatocytes.

Interaction-Trap Assays

A standard yeast two-hybrid assay may be used to assess the effect of a test compound on the MAP kinase-DEF domain interaction (Mendelsohn and Brent, *Curr. Opin. Biotechnol.* 5:482-486, 1994). Typically, a vector encoding a synthetic or naturally occurring peptide containing a DEF domain, covalently bound a DNA binding domain (e.g., GAL4), is transfected into yeast cells containing a reporter gene operably linked to a binding site for the DNA binding domain. Further, a vector encoding either the native MAP kinase of interest, or a synthetic fragment containing the sequence that interacts with the target DEF domain, covalently bound to a transcriptional activator (e.g., GalAD) is also transfected. The effectiveness of a test compound is then assessed by growing the yeast in the presence of the compound and measuring the level of reporter gene expression.

GST Pulldown Assays

The interaction of a MAP kinase with a DEF domain may be examined using a GST-fusion protein binding study. A vector encoding a naturally-occurring or synthetic polypeptide containing the DEF domain of interest is fused to GST and expressed in a host cell (e.g., *E. coli* or *Saccharomyces spp.*). The GST fusion protein is then contacted with a MAP kinase in the presence and absence of a test compound. The MAP kinase may be naturally expressed by the host cell or may be expressed from a second vector inserted into the host cell. Following incubation with the test compound, the host cells are lysed and the GST fusion proteins are recovered using glutathione-Sepharose (GSH-Seph) beads. Typically, the GST fusion proteins are released from the GSH-Seph by

boiling and the proteins visualized by electrophoretic separation on an SDS-PAGE gel. A skilled artisan will readily understand that the GST-Pulldown assay described here can be readily adapted to a cell-free assay by incubating the purified GST fusion protein with a purified recombinant MAP kinase.

Fluorescence Polarization Assay

A variety of well known cell-free techniques may be used to assess the effects of a test compound on the interaction between a MAP kinase and a DEF domain-containing target protein. Fluorescence polarization assays are particularly useful for this purpose. In this assay, a peptide (about 6-12 amino acids) containing a DEF domain at its C-terminus and a fluorophore (e.g., fluorescein) conjugated to its N-terminus is incubated in the presence and absence of increasing amounts of recombinant MAP kinase (e.g., GST-ERK1; 0.01-1 μM) for 10 minutes at room temperature. Aliquots from each reaction are placed in a plate black-walled microtiter (e.g., 384-well) plate and polarization measured using an Analyst plate reader. Increasing concentrations of the MAP kinase causes an increase in polarization. Titrating in the "free" DEF domain-containing peptide (i.e., un-conjugated) inhibits the change in polarization, whereas the mutated DEF domain peptide does not. The appearance of low polarization, even in the presence of high concentrations of kinase, indicates flexible binding of the DEF domain to the kinase and suggests the presence of the propeller effect. Designing shorter dye-conjugated DEF domain-containing peptides usually alleviates this problem. The effect of standard assay variables, including incubation time, temperature, pH (7.2-8.5), and buffers, on polarization is readily controlled during routine assay optimization.

This assay is readily adaptable for identifying test compounds that inhibit binding of a MAP kinase to a DEF domain. The use of automated liquid handling systems and plate readers makes this assay readily adaptable to a high-throughput format for screening large numbers of test compounds. For compound screening, the test compound is added to a mixture of the fluorescently labeled DEF domain-containing peptide and the target

MAP kinase. Compounds that inhibit the polarization increase (or cause a decrease in polarization) resulting from increasing amounts of the MAP kinase are therapeutic candidates.

Identification of Test Compounds as Potential Therapeutics

We have identified a variety of DEF domain-containing target proteins that have been implicated in a variety of diseases. The particular DEF domain or target protein may be substituted for c-Fos in any of the exemplary assays described here. Further, the lists of target proteins provided are not intended to be limiting. Other target proteins are easily identified based on the availability of a DEF domain.

Test compounds having antineoplastic activity are those that inhibit binding of a MAP kinase (e.g., ERK 1/2) to the DEF domain of any of the proteins of Table 1 (except mPer1) or Table 2. Test compounds that are useful for treating cardiovascular disorders inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 3. Test compounds that are useful for treating acute and chronic inflammation or inflammatory disorders inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 4. Test compounds that are useful for treating a variety of metabolic disorders inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 5. Test compounds that are useful for treating a variety of nervous system disorders (e.g., central and peripheral neuropathies) and behavioral disorders (e.g., psychosis, schizophrenia, autism, Down's Syndrome, Parkinson's Disease, Alzheimer's Disease, epilepsy, Cockayne syndrome, depression, and opiate addiction) inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 6. Test compounds useful for treating sleep disorders inhibit MAP kinase binding to the DEF domain of the Per proteins (Table 1: mPer; Table 7). Other potentially useful therapeutics inhibit MAP kinase binding to the DEF domain of the PKA-anchoring proteins (AKAPs) (Table 7). In each of Tables 2-7, the alpha-numeric Accession Codes refer to the SWISS-PROT accession numbers. The numeric Accession Codes refer to the GENPEPT accession numbers. In each case,

the DEF domain is underscored.

Administration of a DEF Domain Inhibitors or Candidate Compounds for the Treatment of Disease

As described above, ERK1/2 activate several IEG products through an interaction with the DEF domain and a subsequent phosphorylation event. It is also well known that activation of certain IEGs, and the proteins identified in Table 2, cause cellular proliferation and may cause tumor promotion and progression. Accordingly, this invention also provides methods and compositions for antineoplastic (i.e., cancer) therapy by administering DEF domain inhibitors. Likewise, therapy for cardiovascular disorders, inflammatory disorders, metabolic disorders, neuropathies and behavioral disorders, and sleep disorders may be provided by inhibiting MAP kinase binding to the DEF domain of one or more of the proteins identified in Table 3, 4, 5, 6, and 7, respectively. Useful DEF domain inhibitors include compounds that bind to the DEF domain of target proteins and prevent the binding of the target kinases. Also, DEF domain inhibitors include "bait" proteins that bind activated target kinases but do not cause cellular proliferation or tumor promotion and/or progression.

In addition to candidate compounds identified using the screening methods of this invention, DEF domain inhibitors can be created by inserting, by artifice, a DEF domain into a non-target protein. The cellular activation/proliferation pathway described herein is limited by the presence of activated target kinase, not by the availability of target proteins. Thus, a DEF domain that is present in a non-target protein effectively "baits" the target kinase, reducing its availability to phosphorylate the target proteins. DEF domains suitable for therapy have the general structure: F/Y—X_1—F/Y—X_2 (SEQ ID NO: 28). Desirably, X_2 is proline. Most desirably, the DEF domain is identical to the DEF domain of the target protein to which therapy is directed. For example, Figures 3B and C demonstrate that the "naked" DEF domains FQFP (SEQ ID NO: 3) and FTYP (SEQ ID NO: 2) are effective inhibitors of target protein phosphorylation. Substitution of

phenylalanine for alanine in these polypeptides results in approximately a two-fold reduction in potency. Accordingly, therapy can be provided by administering pharmaceutical formulations containing a naked DEF domain. Typically, these polypeptides are administered by parenteral injection such as intravenous, intramuscular, or subcutaneous injection. These small polypeptides may be administered in any appropriate formulation including, for example, in a liposomal formulation. The polypeptides may also be injected directly into a solid tumor.

Alternatively, therapy can be achieved by administering a chimeric protein consisting of a DEF domain that is engineered into a non-target protein. Typically, the chimeric protein will "display" the four amino acid DEF domain on a hydrophilic face, making it available to bind to the activated target kinase. The non-target protein can be chosen based upon the desired pharmacokinetic or pharmacodynamic effect and is readily determined by a person of ordinary skill. For example, a DEF domain inhibitor sequence may be engineered into a serum protein such as albumin or ceruloplasmin in order to prolong the plasma half life. Alternatively, the DEF domain may be engineered into a protein that promotes uptake into a particular cell type.

Pharmaceutical Formulations

The peptide agents and candidate compounds of the invention can be administered to a subject, e.g., a human, directly or in combination with any pharmaceutically acceptable carrier or salt known in the art. Pharmaceutically acceptable salts may include non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like. One exemplary pharmaceutically

acceptable carrier is physiological saline. Other physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in Remington's Pharmaceutical Sciences, (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, PA.

Pharmaceutical formulations of a therapeutically effective amount of a peptide agent or candidate compound of the invention, or pharmaceutically acceptable salt-thereof, can be administered orally, parenterally (e.g. intramuscular, intraperitoneal, intravenous, or subcutaneous injection), or by intrathecal or intracerebroventricular injection in an admixture with a pharmaceutically acceptable carrier adapted for the route of administration.

Methods well known in the art for making formulations are found, for example, in Remington's Pharmaceutical Sciences (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, PA. Compositions intended for oral use may be prepared in solid or liquid forms according to any method known to the art for the manufacture of pharmaceutical compositions. The compositions may optionally contain sweetening, flavoring, coloring, perfuming, and/or preserving agents in order to provide a more palatable preparation. Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier or excipient. These may include, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, sucrose, starch, calcium phosphate, sodium phosphate, or kaolin. Binding agents, buffering agents, and/or lubricating agents (e.g., magnesium stearate) may also be used. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and soft gelatin capsules. These forms contain inert diluents commonly used in the art, such as water or an oil medium. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying agents, and suspending agents.

Formulations for parenteral administration (i.e., intravenous, intramuscular, and subcutaneous injection) include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of suitable vehicles include propylene glycol, polyethylene glycol, vegetable oils, gelatin, hydrogenated naphalenes, and injectable organic esters, such as ethyl oleate. Such formulations may also contain adjuvants, such as preserving, wetting, emulsifying, and dispersing agents. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for the proteins of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

Liquid formulations can be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, or by irradiating or heating the compositions. Alternatively, they can also be manufactured in the form of sterile, solid compositions which can be dissolved in sterile water or some other sterile injectable medium immediately before use.

The amount of active ingredient in the compositions of the invention can be varied. One skilled in the art will appreciate that the exact individual dosages may be adjusted somewhat depending upon a variety of factors, including the protein being administered, the time of administration, the route of administration, the nature of the formulation, the rate of excretion, the nature of the subject's conditions, and the age, weight, health, and gender of the patient. Generally, dosage levels of between 0.1 μg/kg to 100 mg/kg of body weight are administered daily as a single dose or divided into multiple doses. Desirably, the general dosage range is between 250 μg/kg to 5.0 mg/kg of body weight per day. Wide variations in the needed dosage are to be expected in view of the differing efficiencies of the various routes of administration. For instance, oral administration generally would be expected to require higher dosage levels than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, which are well known in the art. In general, the

precise therapeutically effective dosage will be determined by the attending physician in consideration of the above identified factors.

The protein or candidate compound of the invention can be administered in a sustained release composition, such as those described in, for example, U.S. Patent No. 5,672,659 and U.S. Patent No. 5,595,760. The use of immediate or sustained release compositions depends on the type of condition being treated and the desired pharmacokinetic profile. For preventive or long-term treatments, a sustained released composition may be preferred.

The protein or candidate compound of the present invention can be prepared in any suitable manner. The protein or candidate compound can be isolated from naturally occurring sources, recombinantly produced, or produced synthetically, or produced by a combination of these methods. The synthesis of short peptides is well known in the art. See e.g. Stewart et al., Solid Phase Peptide Synthesis (Pierce Chemical Co., 2d ed., 1984).

Methods

Cell Culture

NIH 3T3 fibroblasts were transfected with Lipofectamine (Invitrogen, Carlsbad, CA) and then cultured for 18 h in DMEM/10% calf serum. Swiss 3T3 fibroblasts expressing a conditionally active form of B-Raf, were cultured in DMEM containing 10% fetal bovine serum (FBS). Before stimulation, cells were cultured in DMEM containing 20 mM HEPES (starving medium) for 24 h (NIH 3T3 or 208F) or 48 h (Swiss 3T3). Rat-1 fibroblasts were cultured for 48 h, washed with starving medium and culture for an additional 24 h in starving medium. For AP-1 assays, Hela cells were transfected with Lipofectamine for 6 h and then cultured for an additional 16 h before cell lysis and assay of luciferase activity (Promega). EGF and PDGF (Invitrogen) were reconstituted in sterile water containing 0.1% BSA. LPA (Aventi Polar Lipids, Alabaster, AL) was reconstituted in 50% ethanol before sonication for 30 min.

Retroviruses used to infect rat 208F cells were produced as described previously

(Chen *et al., Oncogene*, 12:1493-1502, 1996). Neomycin-resistant pools of c-Fos-expressing cells were assayed for anchorage-independent growth or focus formation. Metabolic labeling with ^{35}S-methionine or ^{32}P-orthophosphate (performed in parallel) was performed as described by Chen *et al.*

Cell lysis and western analysis

Cell extracts were prepared as described previously (Richards, *et al., Curr. Biol.*, 9: 810-820, 1999). To analyze shifts in c-Fos mobility, samples were resolved on a 7.5% SDS-polyacrylamide gel electrophoresis (PAGE) gel, transferred to nitrocellulose and probed with anti-c-Fos antibody (Update Biotechnology Inc., Lake Placid, NY). This antibody is specific for c-Fos and does not cross-react with FosB, Fra-1 or Fra-2. For ERK1/2-MAPK western analysis, a polyclonal anti-ERK1/2 antibody to an anti-phospho-p42/p44 MAPK monoclonal antibody was used (Sigma, St. Louis, MO). Phosphatase treatment of cell extract was performed for 30 min on ice using λ protein phosphatase (New England Biolabs, Beverly, MA).

To generate antiserum specific for phosphorylated Thr 325 in c-Fos, residues 317-329 (VTELEPLCTPVVT) (SEQ ID NO: 20) were synthesized (underlined residue is phospho-Thr at position 325), conjugated to keyhole limpet haemacyanin and injected into rabbits (Research Genetics, Inc., Huntsville, AL). To determine the specificity of the antiserum, extracts from cells expressing vector or c-Fos proteins were immobilized on nitrocellulose and probed with a solution of this anti-serum (1:3000) for 12 h at 4°C.

Recombinant protein purification

M15pREP4 cells transformed with pDS56-$(His)_6$Fos or pETHis$_6$/ERK2 and MEK 1 R4F were cultured at 25°C until an OD_{600} of 0.7 was attained. Cells were then incubated in the presence of 1mM isopropyl-β-D-thiogalactoside (IPTG) for an additional 12 h at 25 ° C. and then harvested by centrifugation. Pellets were resuspended in column buffer (20 mM Tris-HCl at pH 8.0, 200 mM sodium chloride, 10% glycerol, and 10 mM

imidazole) and cells were lysed by passage through a French Press. The $(His)_6$ proteins were purified using Nickel-NTA-agarose resin (Qiagen, Alencia, CA), dialyzed in column buffer containing 50% glycerol and then stored at -20°C.

***In vitro* kinase reactions**

Phosphorylation of $(His)_6$-Fos by ERK1 immunoprecipitated from NIH3T3 cells, activate $(His)_6$-ERK2 (ref. 42) or FLAG-ERK5/BMK1, was performed in kinase buffer containing 10 μCi γ^{32}P-ATP at 30°C (Chen *et al., Mol. Cell. Biol.* 10:3204-3215, 1990). Endogenous ERK1 and RSK kinase activities were performed as described previously (Chung *et al., Mol. Cell. Biol.,* 11:1868-1871, 1991). The HPLC-purified synthetic peptides used in the competition kinase assays were mixed with $(His)_6$-Fos-EE before addition of activated ERK2 and γ-^{32}P-ATP. The peptides derived from ELK-1 were as follows: RRPRSPAKLSFQFPSFQFP (SEQ ID NO: 21); RRPRSPAKLSAQAPSAQAP (SEQ ID NO: 22); RRPRSPAKLSFTYPSFTYP (SEQ ID NO: 23); RRPRSPAKLSATYPSATYP (SEQ ID NO: 24).

Immunofluorescence

Swiss 3T3 cells (1.35×10^5 per 35-mm dish) were cultured on poly-L-lysine-coated glass coverslips for 24 h and serum-starved for 48 h. Cyclohexamide (14 μg/ml) was delivered in dimethyl sulphoxide. After stimulation with growth factors, cells were washed with ice-sold PBS containing 0.1% BSA, fixed with 3.7% formaldehyde for 10 min at room temperature and permeabilized with 0.2% Triton-X100 for 5 min. Analysis of c-Fos expression was performed using a rabbit anti-human c-Fos antibody (1:500, Upstate Biotechnology Inc.) under conditions described by the manufacturer. Conditions for phospho-p24/p44 MAPK immunofluorescence were identical to those used for c-Fos, except that a monoclonal phospho-MAPK antibody was used (Sigma). Coverslips were mounted in Citifluor (Ted Pella Inc., Redding, CA) and examined under epifluorescent illumination.

Bromodeoxyuridine (BrdU) incorporation

Swiss 3T3 cells were cultured as described for immunofluorescence studies, treated with growth factors and 20 μM BrdU Labeling Reagent (Amersham Life Sceinces Inc., Piscataway, NJ) for 20 h at 37 °C. For immunofluorescence analysis, a mouse anti-BrdU monoclonal (Amersham Life Sciences Inc.) supplemented with DNAase I (Invitrogen) was used.

Other Embodiments

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

TABLE 2: ONCOLOGY

	Accession Cod	Target Description	Amino Acid	Target Sequence
1	PIP3_HUMAN	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta 3 (EC 3.1.4.11) (Phosphoinositide phospholipase C) (PLC-beta-3) (Phospholipase C-beta-3).	777	DEEPFDFPKVVLPTL
2	PSD1_HUMAN	26S proteasome non-ATPase regulatory subunit 1 (26S proteasome regulatory subunit S1) (26S proteasome subunit p112).	767	TQFWFWFPLSHFLSL
3	PSD1_HUMAN	26S proteasome non-ATPase regulatory subunit 1 (26S proteasome regulatory subunit S1) (26S proteasome subunit p112).	807	KPSTFAYPAPLEVPK
4	RT31_HUMAN	28S ribosomal protein S31, mitochondrial precursor (S31mt) (MRP-S31) (Imogen 38).	307	EGKLWEFPINNEAGF
5	PDPK_HUMAN	3-phosphoinositide dependent protein kinase-1 (EC 2.7.1.37) (hPDK1).	295	IKLEYDFPEKFFPKA
6	RS3_HUMAN	40S ribosomal protein S3.	73	VQKRFGFPEGSVELY
7	P52K_HUMAN	52 kDa repressor of the inhibitor of the protein kinase (p58IPK- interacting protein) (58 kDa interferon-induced protein kinase- interacting protein) (P52rIPK) (Death associated protein 4).	18	DLAFFRFPRDPARCQ
8	B53A_HUMAN	53 kDa BRG1-associated factor A (Actin-related protein Baf53a) (ArpNbeta).	283	PTVHYEFPNGYNCDF
9	AAK1_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-1 chain (EC 2.7.1.-) (AMPK alpha-1 chain).	273	DLPKYLFPEDPSYSS
10	AAK2_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-2 chain (EC 2.7.1.-) (AMPK alpha-2 chain).	271	DLPSYLFPEDPSYDA
11	RLA0_HUMAN	60S acidic ribosomal protein P0 (L10E).	253	VETDYTFPLAEKVKA
12	RL10_HUMAN	60S ribosomal protein L10 (QM protein) (Tumor suppressor QM) (Laminin receptor homolog).	152	RRAKFKFPGRQKIHI
13	MC3A_MOUSE	80 kda MCM3-associated protein (GANP protein).	184	GLTPFSFPQVTNSSV
14	ASH3_MOUSE	Achaete-scute homolog 3 (bHLH transcriptional regulator Sgn-1) (Mash- 3).	69	DPYPFPFPMPYTNYR
15	ASH3_HUMAN	Achaete-scute homolog 3 (bHLH transcriptional regulator Sgn-1).	69	EPCPFSFPMPYPNYR
16	AD10_HUMAN	ADAM 10 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase domain 10) (Mammalian disintegrin-metalloprotease) (Kuzbanian protein homolog).	286	PTNPFRFPNIGVEKF
17	AD12_HUMAN	ADAM 12 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase domain 12) (Meltrin alpha).	382	ASTGYPFPMVFSSCS
18	BS69_HUMAN	Adenovirus 5 E1A-binding protein (BS69 protein).	229	PDNWFCYPCIPNHEL
19	CYA4_HUMAN	Adenylate cyclase, type IV (EC 4.6.1.1) (ATP pyrophosphate-lyase) (Adenylyl cyclase).	679	ITSLFFFPTSSDCPF
20	AF4_HUMAN	AF-4 protein (Proto-oncogene AF4) (FEL protein).	381	EPSKFPFPTKDSQHV
21	AK11_HUMAN	A-kinase anchor protein 11 (Protein kinase A anchoring protein 11) (PRKA11) (A kinase anchor protein 220 kDa) (AKAP 220) (hAKAP220).	661	EVCQFSYPQTPASPQ
22	AKA3_HUMAN	A-kinase anchor protein 3 (Protein kinase A anchoring protein 3) (PRKA3) (A-kinase anchor protein 110 kDa) (AKAP 110) (Sperm oocyte binding protein) (Fibrousheathin I) (Fibrous sheath protein of 95 kDa) (FSP95).	490	SDISFEYPEDIGNLS
23	ALK_HUMAN	ALK tyrosine kinase receptor precursor (EC 2.7.1.112) (Anaplastic lymphoma kinase) (CD246 antigen).	264	LECSFDFPCELEYSP
24	ANR5_HUMAN	Ankyrin repeat domain protein 5.	437	VIPEYAFPRRQDGGP
25	ATR_HUMAN	Anthrax toxin receptor precursor (Tumor endothelial marker 8).	421	PEQEYEFPEPRNLNN
26	KRAA_HUMAN	A-Raf proto-oncogene serine/threonine-protein kinase (EC 2.7.1.-) (A-raf-1) (Proto-oncogene Pks).	193	DPEHFPFPAPANAPL

27	ABC3_HUMAN	ATP-binding cassette, sub-family A, member 3 (ATP-binding cassette transporter 3) (ATP-binding cassette 3) (ABC-C transporter).	65 LPLFFTFPPPGDTWE
28	KU70_HUMAN	ATP-dependent DNA helicase II, 70 kDa subunit (Lupus Ku autoantigen protein p70) (Ku70) (70 kDa subunit of Ku antigen) (Thyroid-lupus autoantigen) (TLAA) (CTC box binding factor 75 kDa subunit) (CTCBF) (CTC75).	362 RPSLFVYPEESLVIG
29	AXU1_HUMAN	AXIN1 up-regulated gene 1 protein (TGF-beta induced apoptosis protein 3) (TAIP-3) (URAX1 protein).	88 GITVFYFPRCQGFTS
30	12751139	B aggressive lymphoma long isoform [Homo sapiens]	240 SSGIFQFPLNLCTKT
31	11544936	bA271B5.1 (similar to ribosomal protein S7) [Homo sapiens]	183 KDVNFEFPEFQLQTK
32	ACTY_HUMAN	Beta-centractin (Actin-related protein 1B) (ARP1B).	29 QIPKYCFPNYVGRPK
33	BRC1_HUMAN	Breast cancer type 1 susceptibility protein.	825 DTEGFKYPLGHEVNH
34	BA1A_HUMAN	Bromodomain adjacent to zinc finger domain protein 1A (ATP-utilizing chromatin assembly and remodeling factor 1) (hACF1) (ATP-dependent chromatin remodelling protein) (Williams syndrome transcription factor-related chromatin remodeling factor 180) (WCRF18	254 QDFSYFFPDDPPTFI
35	3928855	calcium and DAG-regulated guanine nucleotide exchange factor II [Homo sapiens]	627 EEGPFTFPNGEAVEH
36	KCCA_HUMAN	Calcium/calmodulin-dependent protein kinase type II alpha chain (EC 2.7.1.123) (CaM-kinase II alpha chain) (CaM kinase II alpha subunit) (CaMK-II alpha subunit).	226 KAGAYDFPSPEWDTV
37	CFLA_MOUSE	CASP8 and FADD-like apoptosis regulator precursor (Cellular FLICE-like inhibitory protein) (c-FLIP) (Caspase-eight-related protein) (Casper) (Caspase-like apoptosis regulatory protein) (CLARP) (MACH-related inducer of toxicity) (MRIT) (Caspase homolog) (C	282 HIQLFLFPKSHDITQ
38	CARA_HUMAN	Caspase recruitment domain protein 10 (CARD-containing MAGUK protein 3) (Carma 3).	98 EALEFYYPEHFTLLT
39	CARB_HUMAN	Caspase recruitment domain protein 11 (CARD-containing MAGUK protein 3) (Carma 1).	86 ESLEFYYPELYKLVT
40	CARF_HUMAN	Caspase recruitment domain protein 15 (Nod2 protein) (Inflammatory bowel disease protein 1).	323 QEFLFVFPFSCRQLQ
41	CAR6_HUMAN	Caspase recruitment domain protein 6.	728 LENSWLFPTRIGGNF
42	CTD1_HUMAN	Catenin delta-1 (p120 catenin) (p120(ctn)) (Cadherin-associated Src substrate) (CAS) (p120(cas)).	202 LPRNFHYPPDGYSRH
43	CTD2_HUMAN	Catenin delta-2 (Delta-catenin) (Neural plakophilin-related ARM-repeat protein) (NPRAP) (Neurojungin) (GT24).	162 PEGSFQYPASYHSNQ
44	CEBA_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	27 SSAAFGFPRGAGPAQ
45	CEBA_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	102 GGGDFDYPGAPAGPG
46	CEBE_HUMAN	CCAAT/enhancer binding protein epsilon (C/EBP epsilon).	84 DPRPFAYPPHTFGPD
47	5020264	Cdc42 GTPase-activating protein [Mus musculus]	673 SPAPFPFPEAPGSLP
48	ZIZ1_HUMAN	Cdc42 guanine nucleotide exchange factor zizimin 1.	1457 RSLIYKFPSTFYEGR
49	CBL2_HUMAN	Cdk5 and abl enzyme substrate 2 (Interactor with cdk3 2) (Ik3-2).	241 SYAKFLYPTNALVTH
50	A2M1_HUMAN	Clathrin coat assembly protein AP50 (Clathrin coat associated protein AP50) (Plasma membrane adaptor AP-2 50 kDa protein) (HA2 50 kDa subunit) (Clathrin assembly protein complex 2 medium chain) (AP-2 mu 2 chain).	114 EILDFGYPQNSETGA
51	CTA3_HUMAN	Contactin associated protein-like 3 precursor (Cell recognition molecule Caspr3).	126 EESIWGFPGNTNADS
52	CRKL_HUMAN	Crk-like protein.	128 VRTLYDFPGNDAEDL

53	CRN1_HUMAN	Crooked neck-like protein 1 (Crooked neck homolog) (hCrn) (CGI-201) (MSTP021).	800 EYFDYIFPEDAANQP
54	11385644	CTCL tumor antigen se2-1 [Homo sapiens]	42 TEDDFEFPFAKTNLS
55	SRA4_RAT	CTD-binding SR-like protein RA4 (Fragment).	158 PQAPFGYPGDGMQQP
56	CG1C_HUMAN	Cyclin C.	138 TRFSYAFPKEFPYRM
57	CCT1_HUMAN	Cyclin T1 (Cyclin T) (CycT1).	598 SSLNFSFPSLPTMGQ
58	CPAC_MOUSE	Cytochrome P450 2A12 (EC 1.14.14.1) (CYPIIA12) (Steroid hormones 7- alpha-hydroxylase) (Testosterone 7-alpha-hydroxylase).	456 QNFRFKFPRKLEDIN
59	BMX_HUMAN	Cytoplasmic tyrosine-protein kinase BMX (EC 2.7.1.112) (Bone marrow kinase BMX) (Epithelial and endothelial tyrosine kinase) (ETK) (NTK38).	273 SKISWEFPESSSSEE
60	PA24_HUMAN	Cytosolic phospholipase A2 (CPLA2) [Includes: Phospholipase A2 (EC 3.1.1.4) (Phosphatidylcholine 2-acylhydrolase); Lysophospholipase (EC 3.1.1.5)].	679 STFNFQYPNQAFKRL
61	5NTC_HUMAN	Cytosolic purine 5'-nucleotidase (EC 3.1.3.5) (5'-nucleotidase cytosolic II).	260 MTYLFDFPHGPKPGS
62	1616601	disintegrin-metalloprotease MADM [Homo sapiens]	229 PTNPFRFPNISVEKF
63	DM3A_HUMAN	DNA (cytosine-5)-methyltransferase 3A (EC 2.1.1.37) (Dnmt3a) (DNA methyltransferase HsaIIIA) (DNA MTase HsaIIIA) (M.HsaIIIA).	861 MERVFGFPVHYTDVS
64	DM3B_HUMAN	DNA (cytosine-5)-methyltransferase 3B (EC 2.1.1.37) (Dnmt3b) (DNA methyltransferase HsaIIIB) (DNA MTase HsaIIIB) (M.HsaIIIB).	805 LERIFGFPVHYTDVS
65	DNM2_HUMAN	DNA (cytosine-5)-methyltransferase-like protein 2 (Dnmt2) (DNA methyltransferase homolog HsaIIP) (DNA MTase homolog HsaIIP) (M.HsaIIP) (PuMet).	354 FPPEFGFPEKITVKQ
66	MLH3_HUMAN	DNA mismatch repair protein Mlh3 (MutL protein homolog 3).	1285 LGLEFVFPDTSDSLV
67	DPD2_HUMAN	DNA polymerase delta subunit 2 (EC 2.7.7.7).	389 KTDPFIFPECPHVYF
68	DPE2_HUMAN	DNA polymerase epsilon subunit B (EC 2.7.7.7) (DNA polymerase II subunit B).	236 HVNAFGFPPTEPSST
69	DPG2_HUMAN	DNA polymerase gamma subunit 2, mitochondrial precursor (EC 2.7.7.7) (Mitochondrial DNA polymerase accessory subunit) (PolG-beta) (MtPolB) (DNA polymerase gamma accessory 55 kDa subunit) (p55).	287 NKLYYNFPWGKELIE
70	TP2A_MOUSE	DNA topoisomerase II, alpha isozyme (EC 5.99.1.3).	633 HRIQFKYPGPEDDAA
71	TP2B_HUMAN	DNA topoisomerase II, beta isozyme (EC 5.99.1.3).	1444 FGNLFSFPSYSQKSE
72	STAU_HUMAN	Double-stranded RNA-binding protein Staufen homolog.	114 PRYFYPFPVPPLLYQ
73	MPK4_HUMAN	Dual specificity mitogen-activated protein kinase kinase 4 (EC 2.7.1.-) (MAP kinase kinase 4) (JNK activating kinase 1) (c-Jun N- terminal kinase kinase 1) (JNKK) (SAPK/ERK kinase 1) (SEK1).	301 ATGRFPYPKWNSVFD
74	DUS1_HUMAN	Dual specificity protein phosphatase 1 (EC 3.1.3.48) (EC 3.1.3.16) (MAP kinase phosphatase-1) (MKP-1) (Protein-tyrosine phosphatase CL100) (Dual specificity protein phosphatase hVH1).	335 TTTVFNFPVSIPVHS
75	DUS4_HUMAN	Dual specificity protein phosphatase 4 (EC 3.1.3.48) (EC 3.1.3.16) (Mitogen-activated protein kinase phosphatase-2) (MAP kinase phosphatase-2) (MKP-2) (Dual specificity protein phosphatase hVH2).	362 SQFVFSFPVSVGVHS
76	EDA_HUMAN	Ectodysplasin A (Ectodermal dysplasia protein) (EDA protein).	134 ALLNFFFPDEKPYSE

77	NPP2_HUMAN	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (E-NPP 2) (Phosphodiesterase I/nucleotide pyrophosphatase 2) (Phosphodiesterase I alpha) (PD-Ialpha) (Autotaxin) [Includes: Alkaline phosphodiesterase I (EC 3.1.4.1); Nucleotide pyrophosphatase (EC 3.6.1.	676 MSYGFLFPPYLSSSP
78	EF1G_HUMAN	Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) (PRO1608).	322 WYSEYRFPEELTQTF
79	GAT2_HUMAN	Endothelial transcription factor GATA-2.	167 GSHLFGFPPTPPKEV
80	CASL_HUMAN	Enhancer of filmentation 1 (HEF1) (CRK-associated substrate-related protein) (CAS-L) (CasL) (PP105) (Neural precursor cell expressed developmentally down-regulated 9).	237 REKDYDFPPPMRQAG
81	ESR1_MOUSE	Estrogen receptor (ER) (Estradiol receptor) (ER-alpha).	48 KPTVFNYPEGAAYEF
82	ETV2_HUMAN	Ets translocation variant 2 (Ets-related protein 71).	25 AKLGFCFPDLALQGD
83	ELK3_HUMAN	ETS-domain protein ELK-3 (ETS-related protein NET) (ETS-related protein ERP) (SRF accessory protein 2) (SAP-2).	370 PSTLFQFPTLLNGHM
84	ELK4_HUMAN	ETS-domain protein ELK-4 (Serum response factor accessory protein 1) (SAP-1).	394 ANTLFQFPSVLNSHG
85	ERF_HUMAN	ETS-domain transcription factor ERF (Ets2 repressor factor).	138 GGSHFRFPPSTPSEV
86	ERF_HUMAN	ETS-domain transcription factor ERF (Ets2 repressor factor).	5 ADTGFAFPDWAYKPE
87	ETV3_HUMAN	ETS-related protein PE-1 (ETS translocation variant 3) (Fragment).	168 ASSRFHFPPLDTHSP
88	ETV3_HUMAN	ETS-related protein PE-1 (ETS translocation variant 3) (Fragment).	35 GGGGYQFPDWAYKTE
89	IF33_HUMAN	Eukaryotic translation initiation factor 3 subunit 3 (eIF-3 gamma) (eIF3 p40 subunit) (eIF3h).	78 ITNCFPFPQHTEDDA
90	IF37_HUMAN	Eukaryotic translation initiation factor 3 subunit 7 (eIF-3 zeta) (eIF3 p66) (eIF3d).	331 GKERYNFPNPNPFVE
91	FOL1_HUMAN	Folate receptor alpha precursor (FR-alpha) (Folate receptor 1) (Folate receptor, adult) (Adult folate-binding protein) (FBP) (Ovarian tumor- associated antigen MOv18) (KB cells FBP).	176 QPFHFYFPTPTVLCN
92	FXJ2_HUMAN	Forkhead box protein J2 (Fork head homologous X).	404 NNTGFAFPSDWCSNI
93	FXK1_MOUSE	Forkhead box protein K1 (Myocyte nuclear factor) (MNF).	170 QQCTFRFPSTAIKIQ
94	FZD4_HUMAN	Frizzled 4 precursor (Frizzled-4) (Fz-4) (hFz4) (FzE4).	244 DSSRFSYPERPIIFL
95	GCP6_HUMAN	Gamma-tubulin complex component 6 (GCP-6).	1451 LPRAFAFPVDPQVQS
96	GGPP_HUMAN	Geranylgeranyl pyrophosphate synthetase (GGPP synthetase) (GGPPSASE) (Geranylgeranyl diphosphate synthase) [Includes: Dimethylallyltransferase (EC 2.5.1.1); Geranyltranstransferase (EC 2.5.1.10); Farnesyltranstransferase (EC 2.5.1.29)].	209 TEGKFSFPTIHAIWS
97	KG3A_HUMAN	Glycogen synthase kinase-3 alpha (EC 2.7.1.37) (GSK-3 alpha).	350 NYTEFKFPQIKAHPW
98	GDF3_HUMAN	Growth/differentiation factor 3 precursor (GDF-3).	88 DQGFFLYPKKISQAS
99	4206785	guanine nucleotide-binding protein [Mus musculus]	104 NLPNFDFPPEFYEHA
100	GBAS_HUMAN	Guanine nucleotide-binding protein G(S), alpha subunit (Adenylate cyclase-stimulating G alpha protein).	136 NVPDFDFPPEFYEHA
101	HM21_HUMAN	High-mobility group protein 2-like 1 (HMGBCG protein).	207 DEESFQYPSQQATVK
102	4151328	high-risk human papilloma viruses E6 oncoproteins targeted protein E6TP1 alpha; putative GAP protein alpha [Homo sapiens]	1054 VSYEFKFPFRNNNKW
103	HDA3_HUMAN	Histone deacetylase 3 (HD3) (RPD3-2).	194 KYGNYFFPGTGDMYE
104	DBX1_MOUSE	Homeobox protein DBX1.	133 PPKTFAFPYFEGSFQ
105	HXCC_HUMAN	Homeobox protein Hox-C12 (Hox-3F).	20 TGDTFYFPNFRASGA
106	HMPH_HUMAN	Homeobox protein PRH (Hematopoietically expressed homeobox) (Homeobox protein HEX).	97 GGPLYPFPRTVNDYT
107	HIK2_HUMAN	Homeodomain-interacting protein kinase 2 (EC 2.7.1.-).	1067 AQAPYSFPHNSPSHG

108	IKAP_HUMAN	IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150).	599 FPVRFPYPCTQTELA
109	IP3L_HUMAN	Inositol-trisphosphate 3-kinase B (EC 2.7.1.127) (Inositol 1,4,5- trisphosphate 3-kinase) (IP3K) (IP3 3-kinase) (IP3K-B).	52 RGASFLFPPAESLSP
110	ITA5_HUMAN	Integrin alpha-5 precursor (Fibronectin receptor alpha subunit) (Integrin alpha-F) (VLA-5) (CD49e).	422 QGVVFVFPGGPGGLG
111	MM01_HUMAN	Interstitial collagenase precursor (EC 3.4.24.7) (Matrix metalloproteinase-1) (MMP-1) (Fibroblast collagenase).	364 IYSSFGFPRTVKHID
112	13383464	kinesin-related protein HASH [Mus musculus]	46 NTRDFMFPGPNQMSG
113	KLF4_MOUSE	Kruppel-like factor 4 (Gut enriched kruppel-like factor) (Epithelial zinc-finger protein EZF).	134 STCSFSYPIRAGGDP
114	LAF4_HUMAN	LAF-4 protein (Lymphoid nuclear protein related to AF4).	325 EPTKFPFPNKDSQLV
115	LAMA_HUMAN	Lamin A/C (70 kDa lamin).	477 PLLTYRFPPKFTLKA
116	4324434	large tumor suppressor 1 [Homo sapiens]	334 SSSKFNFPSGRPGMQ
117	930341	LAR-interacting protein 1a [Homo sapiens]	530 SVPDFRFPMADGHTD
118	EDG2_HUMAN	Lysophosphatidic acid receptor Edg-2 (LPA receptor 1) (LPA-1).	76 VNRRFHFPIYYLMAN
119	EDG7_HUMAN	Lysophosphatidic acid receptor Edg-7 (LPA receptor 3) (LPA-3).	57 KNRKFHFPFYYLLAN
120	MM12_HUMAN	Macrophage metalloelastase precursor (EC 3.4.24.65) (HME) (Matrix metalloproteinase-12) (MMP-12) (Macrophage elastase) (ME).	367 SIHSFGFPNFVKKID
121	MKK2_HUMAN	MAP kinase-activated protein kinase 2 (EC 2.7.1.-) (MAPK-activated protein kinase 2) (MAPKAP kinase 2) (MAPKAPK-2).	280 RMGQYEFPNPEWSEV
122	MAPE_HUMAN	Melanoma antigen preferentially expressed in tumors (Preferentially expressed antigen of melanoma) (OPA-interacting protein 4) (OIP4).	140 RASLYSFPEPEAAQP
123	MAG3_HUMAN	Melanoma-associated antigen 3 (MAGE-3 antigen) (Antigen MZ2-D).	141 GNWQYFFPVIFSKAS
124	TIM3_HUMAN	Metalloproteinase inhibitor 3 precursor (TIMP-3) (Tissue inhibitor of metalloproteinases-3) (MIG-5 protein).	168 MLSNFGYPGYQSKHY
125	AMP1_HUMAN	Methionine aminopeptidase 1 (EC 3.4.11.18) (MetAP 1) (Peptidase M 1) (Fragment).	191 PLNYYNFPKSCCTSV
126	MAPA_HUMAN	Microtubule-associated protein 1A (MAP 1A) (Proliferation-related protein p80) [Contains: MAP1 light chain LC2].	37 KPCCYIFPGGRGDSA
127	RT29_HUMAN	Mitochondrial 28S ribosomal protein S29 (S29mt) (MRP-S29) (Death- associated protein 3) (DAP-3) (Ionizing radiation resistance conferring protein).	113 KNTSFAYPAIRYLLY
128	3133291	mitogen activated protein kinase activated protein kinase [Homo sapiens]	259 MTGSFEFPEEEWSQI
129	MK06_HUMAN	Mitogen-activated protein kinase 6 (EC 2.7.1.-) (Extracellular signal- regulated kinase 3) (ERK-3) (MAP kinase isoform p97) (p97-MAPK).	315 YMSIYSFPMDEPISS
130	M3K6_HUMAN	Mitogen-activated protein kinase kinase kinase 6 (EC 2.7.1.-).	800 HRPLFAFPDAVKQIL
131	TAB1_HUMAN	Mitogen-activated protein kinase kinase kinase 7 interacting protein 1 (TAK1-binding protein 1).	361 LVRNFGYPLGEMSQP
132	M4K2_HUMAN	Mitogen-activated protein kinase kinase kinase kinase 2 (EC 2.7.1.37) (MAPK/ERK kinase kinase kinase 2) (MEK kinase kinase 2) (MEKKK 2) (Germinal center kinase) (GC kinase) (Rab8 interacting protein) (B lymphocyte serine/threonine protein kinase).	737 PELTFDFPIETVVCL
133	MDR1_HUMAN	Multidrug resistance protein 1 (P-glycoprotein 1) (CD243 antigen).	1038 GEVVFNYPTRPDIPV
134	MDR1_HUMAN	Multidrug resistance protein 1 (P-glycoprotein 1) (CD243 antigen).	395 RNVHFSYPSRKEVKI

135	MDR3_HUMAN	Multidrug resistance protein 3 (P-glycoprotein 3).	88 TAGNFSFPVNFSLSL
136	MDR3_HUMAN	Multidrug resistance protein 3 (P-glycoprotein 3).	397 NDVHFSYPSRANVKI
137	DRNL_HUMAN	Muscle-specific DNase I-like precursor (EC 3.1.21.-) (DNase X) (XIB).	242 TAAAFDFPTSFQLTE
138	6959304	MYB-binding protein 1A [Homo sapiens]	497 TKHPFSFPLENQARE
139	2706549	myc-intron-binding protein-1 [Mus musculus]	135 SEDLFPFPMHGHSGG
140	MY15_HUMAN	Myosin XV (Unconventional myosin-15).	751 RGAAFGFPGASPRAS
141	MM08_HUMAN	Neutrophil collagenase precursor (EC 3.4.24.34) (Matrix metalloproteinase-8) (MMP-8) (PMNL collagenase) (PMNL-CL).	364 DISNYGFPSSVQAID
142	KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP- 1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	405 SFPHYGFPTYGGITF
143	KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP- 1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	400 TGPGYSFPHYGFPTY
144	RI14_HUMAN	Nuclear factor RIP140 (Nuclear receptor interacting protein 1).	996 DNRTFSYPGVVKTPV
145	RI14_HUMAN	Nuclear factor RIP140 (Nuclear receptor interacting protein 1).	905 NDLEFKYPAGHGSAS
146	N153_HUMAN	Nuclear pore complex protein Nup153 (Nucleoporin Nup153) (153 kDa nucleoporin).	421 RESGFSYPNFSLPAA
147	NCR1_HUMAN	Nuclear receptor co-repressor 1 (N-CoR1) (N-CoR).	23 HSVQYTFPNTRHQQE
148	RORG_HUMAN	Nuclear receptor ROR-gamma (Nuclear receptor RZR-gamma).	540 PPSPFSFPMNPGGWS
149	DD21_HUMAN	Nucleolar RNA helicase II (Nucleolar RNA helicase Gu) (RH II/Gu) (DEAD-box protein 21).	136 RGVTFLFPIQAKTFH
150	NDK5_HUMAN	Nucleoside diphosphate kinase homolog 5 (NDK-H 5) (NDP kinase homolog 5) (nm23-H5) (Testis-specific nm23 homolog) (Inhibitor of p53-induced apoptosis-beta) (IPIA-beta).	137 REIRFMFPEVIVEPI
151	OXE2_HUMAN	Olfactory receptor 51E2 (Prostate specific G-protein coupled receptor) (HPRAJ).	150 RGSLFFFPLPLLIKR
152	ORC4_HUMAN	Origin recognition complex subunit 4.	217 LMNSFGFPQYVKIFK
153	ORC5_HUMAN	Origin recognition complex subunit 5.	24 ERHHFSFPSIFIYGH
154	2645205	p160 myb-binding protein [Mus musculus]	495 TKQHFSFPLDDRNRG
155	PAXI_HUMAN	Paxillin.	114 EEHVYSFPNKQKSAE
156	CYP6_HUMAN	Peptidyl-prolyl cis-trans isomerase like 2 (EC 5.2.1.8) (PPIase) (Rotamase) (Cyclophilin-60) (Cyclophilin-like protein Cyp-60).	45 SLQPFVYPVCTPDGI
157	CSKP_HUMAN	Peripheral plasma membrane protein CASK (EC 2.7.1.-) (hCASK) (Calcium/calmodulin-dependent serine protein kinase) (Lin-2 homolog).	763 HPDRFAYPIPHTTRP
158	PECI_HUMAN	Peroxisomal 3,2-trans-enoyl-CoA isomerase (EC 5.3.3.8) (Dodecenoyl-CoA delta-isomerase) (D3,D2-enoyl-CoA isomerase) (DBI-related protein 1) (DRS-1) (Hepatocellular carcinoma-associated antigen 88).	246 GCSSYTFPKIMSPAK
159	PPAR_HUMAN	Peroxisome proliferator activated receptor alpha (PPAR-alpha).	417 PDDIFLFPKLLQKMA
160	PPAS_MOUSE	Peroxisome proliferator activated receptor delta (PPAR-delta) (PPAR-beta) (Nuclear hormone receptor 1) (NUC1).	389 PDSQYLFPKLLQKMA
161	P85A_MOUSE	Phosphatidylinositol 3-kinase regulatory alpha subunit (PI3-kinase p85-alpha subunit) (PtdIns-3-kinase p85-alpha) (PI3K).	269 SPVLFRFPAASSDNT
162	PI4K_HUMAN	Phosphatidylinositol 4-kinase alpha (EC 2.7.1.67) (PI4-kinase) (PtdIns-4-kinase) (PI4K-alpha).	628 GLDLFVFPYRVVATA
163	PTEN_HUMAN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase PTEN (EC 3.1.3.67) (Mutated in multiple advanced cancers 1).	237 KFMYFEFPQPLPVCG

164	P11B_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, beta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit beta) (PtdIns-3-kinase p110) (PI3K) (PI3Kbeta).	608 ELLDFNYPDQYVREY
165	P11D_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, delta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit delta) (PtdIns- 3-kinase p110) (PI3K) (p110delta).	581 ELLDFSFPDCHVGSF
166	P11D_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, delta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit delta) (PtdIns- 3-kinase p110) (PI3K) (p110delta).	161 AWLQYSFPLQLEPSA
167	940231	phosphodiesterase A' subunit [Homo sapiens]	366 ADEYFTFPKGPVDET
168	22758919	phosphoinositide 3 kinase P110delta [Mus musculus]	580 ELLDFSFPDCYVGSF
169	2696236	phospholipase B [Rattus norvegicus]	42 TLKNFSFPCKPKKLE
170	PLEK_HUMAN	Pleckstrin (Platelet p47 protein).	213 PDAFYYFPDSGFFCE
171	3002588	Plenty of SH3s; POSH [Mus musculus]	376 TGPAFTFPSDVPYQA
172	PAB5_HUMAN	Polyadenylate-binding protein 5 (Poly(A)-binding protein 5) (PABP 5).	176 YVGRFKFPEERAAEV
173	PAB5_HUMAN	Polyadenylate-binding protein 5 (Poly(A)-binding protein 5) (PABP 5).	62 GYVNFRFPADAEWAL
174	15667468	polyploidy associated protein kinase PAPK-A [Mus musculus]	402 SELEFQFPDDKDPVW
175	DP1_HUMAN	Polyposis locus protein 1 (TB2 protein).	56 NLIGFGYPAYISIKA
176	CIQ3_HUMAN	Potassium voltage-gated channel subfamily KQT member 3 (Potassium channel KQT-like 3).	587 KGSAFTFPSQQSPRN
177	MV10_HUMAN	Potentail helicase MOV-10.	601 KKGEYVFPAKKKLQE
178	A11A_HUMAN	Potential phospholipid-transporting ATPase IS (EC 3.6.3.1) (Fragment).	552 KNVCFIFPQFLYQFF
179	4097902	potential transcriptional repressor Not4hp [Mus musculus]	485 VYNSFGFPGQAARYP
180	PRD2_HUMAN	PR-domain zinc finger protein 2 (Retinoblastoma protein-interacting zinc-finger protein) (MTE-binding protein) (MTB-ZF).	354 VFETFMFPCQHCERK
181	DDX6_HUMAN	Probable ATP-dependent RNA helicase p54 (Oncogene RCK) (DEAD-box protein 6).	405 VVINFDFPKLAETYL
182	GPR4_HUMAN	Probable G protein-coupled receptor GPR4 (GPR19).	186 VFVGFLFPWALMLLS
183	GP68_HUMAN	Probable G protein-coupled receptor GPR68 (Ovarian cancer G protein- coupled receptor 1) (OGR-1).	190 FLVGFLFPICLLLAS
184	K682_HUMAN	Probable RNA-binding protein KIAA0682.	446 AFITFMFPEHAVKAY
185	MN1_HUMAN	Probable tumor suppressor protein MN1.	455 QEARFDFPGSAGVDR
186	FAFX_HUMAN	Probable ubiquitin carboxyl-terminal hydrolase FAF-X (EC 3.1.2.15) (Ubiquitin thiolesterase FAF-X) (Ubiquitin-specific processing protease FAF-X) (Deubiquitinating enzyme FAF-X) (Fat facets protein related, X-linked) (Ubiquitin-specific protease 9, X chro	1805 FNDYFEFPRELDMEP
187	FAFY_HUMAN	Probable ubiquitin carboxyl-terminal hydrolase FAF-Y (EC 3.1.2.15) (Ubiquitin thiolesterase FAF-Y) (Ubiquitin-specific processing protease FAF-Y) (Deubiquitinating enzyme FAF-Y) (Fat facets protein related, Y-linked) (Ubiquitin-specific protease 9, Y chro	1616 RDDVFGYPHQFEDKP
188	PDC2_HUMAN	Programmed cell death protein 2 (Zinc finger protein Rp-8).	183 PDHNFLFPEFEIVIE
189	3858885	proliferation potential-related protein [Mus musculus]	742 TDTLFVFPSREDATP
190	KPCM_HUMAN	Protein kinase C, mu type (EC 2.7.1.-) (nPKC-mu) (Protein kinase D).	794 QNAAFMYPPNPWKEI
191	KPCN_HUMAN	Protein kinase C, nu type (EC 2.7.1.-) (nPKC-nu) (Protein kinase EPK2).	787 QNAAFMYPPNPWREI
192	15080775	protein kinase NYD-SP5 [Homo sapiens]	97 DQKSFIFPQESEGTF
193	PP3D_HUMAN	Protein phosphatase 1, regulatory subunit 3D (Protein phosphatase 1, regulatory subunit 6) (Protein phosphatase 1 binding subunit R6).	234 DVFTFGFPVPPFLLE

194	10567793	protein tyrosine phosphatase BK [Mus musculus]	262	RDRRFHFPEETPETP
195	1144002	protein tyrosine phosphatase D30 [Rattus norvegicus]	261	RDRPFHFPEETPETP
196	23268287	protein tyrosine phosphatase receptor-like protein J [Mus musculus]	172	TNNSFAFPESNETQA
197	PTND_HUMAN	Protein tyrosine phosphatase, non-receptor type 13 (EC 3.1.3.48) (Protein-tyrosine phosphatase 1E) (PTP-E1) (hPTPE1) (PTP-BAS) (Protein-tyrosine phosphatase PTPL1) (Fas-associated protein-tyrosine phosphatase 1) (FAP-1).	1704	ICTMFYYPQKIPNKP
198	PAR2_HUMAN	Proteinase activated receptor 2 precursor (PAR-2) (Thrombin receptor- like 1) (Coagulation factor II receptor-like 1).	247	AIGVFLFPAFLTASA
199	PTPJ_MOUSE	Protein-tyrosine phosphatase eta precursor (EC 3.1.3.48) (R-PTP-eta) (HPTP beta-like tyrosine phosphatase).	172	TNNTFAFPESNETQA
200	PTPZ_RAT	Protein-tyrosine phosphatase zeta precursor (EC 3.1.3.48) (R-PTP- zeta) (Phosphacan) (3F8 chondroitin sulfate proteoglycan) (3H1 keratan sulfate proteoglycan).	1565	TSTDFSFPDVNEKDA
201	PTPZ_RAT	Protein-tyrosine phosphatase zeta precursor (EC 3.1.3.48) (R-PTP- zeta) (Phosphacan) (3F8 chondroitin sulfate proteoglycan) (3H1 keratan sulfate proteoglycan).	720	HYSTFAYPPTEVTSH
202	FAT2_HUMAN	Protocadherin Fat 2 precursor (hFat2) (Multiple epidermal growth factor-like domains 1).	4222	LYGGFPFPLEMENKR
203	8216989	putative cell cycle control protein [Homo sapiens]	100	NNQLFRFPATSPLKT
204	GP40_HUMAN	Putative G protein-coupled receptor GPR40.	244	NVASFLYPNLGGSWR
205	RBM9_HUMAN	Putative RNA-binding protein 9 (RNA binding motif protein 9).	251	IIPGFPYPTAATTAA
206	6007826	rab escort protein-2 [Mus musculus]	370	GNTPFIFPLYGHGEI
207	RAE1_HUMAN	Rab proteins geranylgeranyltransferase component A 1 (Rab escort protein 1) (REP-1) (Choroideraemia protein) (TCD protein).	369	GNTPFLFPLYGQGEL
208	RAE2_HUMAN	Rab proteins geranylgeranyltransferase component A 2 (Rab escort protein 2) (REP-2) (Choroideraemia-like protein).	371	GNTPFLFPLYGQGEI
209	RBPL_HUMAN	Recombining binding protein suppressor of hairless-like protein (Transcription factor RBP-L).	311	HKCAFQFPGSPPGGG
210	RB_HUMAN	Retinoblastoma-associated protein (PP110) (P105-RB) (RB).	786	PRSPYKFPSSPLRIP
211	RBB5_HUMAN	Retinoblastoma-binding protein 5 (RBBP-5) (Retinoblastoma-binding protein RBQ-3).	100	CDQRFRFPSPILKVQ
212	RBB8_HUMAN	Retinoblastoma-binding protein 8 (RBBP-8) (CtBP interacting protein) (CtIP) (Retinoblastoma-interacting protein and myosin-like) (RIM).	467	KENAFPFPMDNQFSM
213	RBL2_HUMAN	Retinoblastoma-like protein 2 (130 kDa retinoblastoma-associated protein) (PRB2) (P130) (RBR-2).	170	FQDIFKYPQEEQPRQ
214	12053793	retinoid-acid induced protein 1 [Homo sapiens]	1830	PISLFSFPPLLPQQF
215	ARH2_HUMAN	Rho guanine nucleotide exchange factor 2 (GEF-H1 protein) (Proliferating cell nucleolar antigen p40).	505	KDQKYIFPTLDKPSV
216	RHG7_HUMAN	Rho-GTPase-activating protein 7 (Rho-type GTPase-activating protein 7) (Deleted in liver cancer 1 protein) (Dlc-1) (HP protein) (StAR-related lipid transfer protein 12) (StARD12) (START domain-containing protein 12).	34	LYEDFLFPIDISLVK
217	RBMS_HUMAN	RNA-binding protein with multiple splicing (RBP-MS).	163	PPPAFTYPASLHAQM
218	RUN2_HUMAN	Runt-related transcription factor 2 (Core-binding factor, alpha 1 subunit) (CBF-alpha 1) (Acute myeloid leukemia 3 protein) (Oncogene AML-3) (Polyomavirus enhancer binding protein 2 alpha A subunit) (PEBP2-alpha A) (PEA2-alpha A) (SL3-3 enhancer factor 1	450	SSGSYQFPMVPGGDR

219	SEN2_HUMAN	Sentrin-specific protease 2 (EC 3.4.22.-) (Sentrin/SUMO-specific protease SENP2).	66 AASLFGFPFQLTTKP
220	SEN2_HUMAN	Sentrin-specific protease 2 (EC 3.4.22.-) (Sentrin/SUMO-specific protease SENP2).	437 VFSTFFYPKLKSGGY
221	SEP1_HUMAN	Septin 1 (LARP) (Serologically defined breast cancer antigen NY-BR- 24).	195 EIHIYQFPECDSDED
222	SEP4_HUMAN	Septin 4 (Peanut-like protein 2) (Brain protein H5) (Cell division control-related protein 2) (hCDCREL-2) (Bradeion beta) (CE5B3 beta) (Cerebral protein-7) (hucep-7).	314 GIKIYQFPDCDSDED 214 GIHVYQFPECDSDED
223	SEP5_HUMAN	Septin 5 (Peanut-like protein 1) (Cell division control related protein 1) (CDCREL-1).	200 KIKIYEFPETDDEEE
224	SEP7_HUMAN	Septin 7 (CDC10 protein homolog).	
225	STK6_HUMAN	Serine/threonine kinase 6 (EC 2.7.1.37) (Serine/threonine kinase 15) (Aurora/IPL1-related kinase 1) (Aurora-related kinase 1) (hARK1) (Aurora-A) (Breast-tumor-amplified kinase).	342 SRVEFTFPDFVTEGA 225 RQVDFKFPSSVPAGA
226	STKD_MOUSE	Serine/threonine protein kinase 13 (EC 2.7.1.37) (Aurora/Ipl1/Eg2 protein 1).	371 EFRTYSFPCYLPQPL
227	KPT3_HUMAN	Serine/threonine protein kinase PCTAIRE-3 (EC 2.7.1.-).	
228	2ACA_HUMAN	Serine/threonine protein phosphatase 2A, 72/130 kDa regulatory subunit B (PP2A, subunit B, B"-PR72/PR130) (PP2A, subunit B, B72/B130 isoforms) (PP2A, subunit B, PR72/PR130 isoforms) (PP2A, subunit B, R3 isoform).	708 NIPRFYFPEGLPDTC 96 SLHSYPFPGTIKSRD
229	ST19_HUMAN	Serine/threonine-protein kinase 19 (EC 2.7.1.37) (RP1 protein) (G11 protein).	
230	MAK_MOUSE	Serine/threonine-protein kinase MAK (EC 2.7.1.-) (Male germ cell- associated kinase) (Protein kinase RCK).	432 KESPFRFPDSGLPVS 232 SSMNFRFPQCVPINL
231	MAK_HUMAN	Serine/threonine-protein kinase MAK (EC 2.7.1.-) (Male germ cell- associated kinase).	421 EFKNYNFPKYKPEPL
232	KPT2_HUMAN	Serine/threonine-protein kinase PCTAIRE-2 (EC 2.7.1.-).	627 AVPSFDFPKTPSSQN
233	ULK1_HUMAN	Serine/threonine-protein kinase ULK1 (EC 2.7.1.-) (Unc-51-like kinase 1).	664 SYLIYVFPDRPKDEV
234	ST5A_HUMAN	Signal transducer and activator of transcription 5A.	664 NYLIYVFPDRPKDEV
235	ST5B_HUMAN	Signal transducer and activator of transcription 5B.	333 REGFYLFPDGRNQNP
236	CBL_HUMAN	Signal transduction protein CBL (Proto-oncogene c-CBL).	966 ILREFAFPPPVSPRL
237	CBLB_HUMAN	Signal transduction protein CBL-B (SH3-binding protein CBL-B).	2225 SSSSFPFPCKAWPSG
238	6649242	splicing coactivator subunit SRm300 [Homo sapiens]	
239	S3B3_HUMAN	Splicing factor 3B subunit 3 (Spliceosome associated protein 130) (SAP 130) (SF3b130) (Pre-mRNA splicing factor SF3b 130 kDa subunit).	1162 SFRSYYFPVKNVIDG 253 SNSVFTYPENGTDDF
240	STAC_HUMAN	Stac protein (SRC homology 3 and cysteine-rich domain protein).	186 GVKAFSFPETVFTTV
241	TX18_HUMAN	T-box transcription factor TBX18 (T-box protein 18) (Fragment).	205 EFRTFIFPETVFTAV
242	TX20_HUMAN	T-box transcription factor TBX20 (T-box protein 20) (Fragment).	597 FGSLFPYPYTYMAAA
243	TBX3_HUMAN	T-box transcription factor TBX3 (T-box protein 3).	235 GMASFRFPETTFISV
244	TBX6_HUMAN	T-box transcription factor TBX6 (T-box protein 6).	106 SSANFTFPGYPIHVP
245	T2AY_HUMAN	TFIIA-alpha and beta like factor (ALF).	
246	TFH3_HUMAN	TFIIH basal transcription factor complex p34 subunit (Basic transcription factor 2 34 kDa subunit) (BTF2-p34) (General transcription factor IIH polypeptide 3).	65 QESRFLYPGKNGRLG 270 NAKEFIFPNMQGQGS
247	TOB1_HUMAN	Tob1 protein (Transducer of erbB-2 1).	147 SPHLFTFPPTPPKDV
248	GAT3_HUMAN	Trans-acting T-cell specific transcription factor GATA-3.	104 VSPRFSFPGTTGSLA
249	GAT4_HUMAN	Transcription factor GATA-4 (GATA binding factor-4).	365 EPEDFAFPSTAPSPQ
250	GAT5_HUMAN	Transcription factor GATA-5 (GATA binding factor-5).	345 APQPYTFPASLSTIN
251	TF65_MOUSE	Transcription factor p65 (Nuclear factor NF-kappa-B p65 subunit).	

252	TCL5_HUMAN	Transcription factor-like 5 protein (Cha transcription factor) (HPV-16 E2 binding protein 1) (E2BP-1).	203	TALQFTYPLFTTNAC
253	TF1A_HUMAN	Transcription intermediary factor 1-alpha (TIF1-alpha) (Tripartite motif protein 24).	729	PPENYDFPVVIVKQE
254	ERG_MOUSE	Transcriptional regulator ERG (Fragment).	52	GGAAFIFPNTSVYPE
255	TERA_HUMAN	Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)- ATPase p97 subunit) (Valosin containing protein) (VCP) [Contains: Valosin].	767	GFGSFRFPSGNQGGA
			220	GLRRFAFPLSLFQGS
256	E2BE_HUMAN	Translation initiation factor eIF-2B epsilon subunit (eIF-2B GDP-GTP exchange factor).		
257	SSRA_HUMAN	Translocon-associated protein, alpha subunit precursor (TRAP-alpha) (Signal sequence receptor alpha subunit) (SSR-alpha).	120	LDASFRYPQDYQFYI
			2437	RPGSFTFPGDSDSLQ
258	TRIO_HUMAN	Triple functional domain protein (PTPRF interacting protein).	947	SKDKFEFPLTPVGEE
259	1113923	tyrosine phosphatase-like protein IA-2a [Rattus norvegicus]		
260	BTK_HUMAN	Tyrosine-protein kinase BTK (EC 2.7.1.112) (Bruton's tyrosine kinase) (Agammaglobulinaemia tyrosine kinase) (ATK) (B cell progenitor kinase) (BPK).	94	IIERFPYPFQVVYDE
			114	YRIRFYFPRWYCSGS
261	JAK2_HUMAN	Tyrosine-protein kinase JAK2 (EC 2.7.1.112) (Janus kinase 2) (JAK-2).		
262	JAK3_HUMAN	Tyrosine-protein kinase JAK3 (EC 2.7.1.112) (Janus kinase 3) (JAK-3) (Leukocyte janus kinase) (L-JAK).	100	YRIRFYFPNWFGLEK
			292	RIKSYSFPKPGHRKS
263	KSYK_HUMAN	Tyrosine-protein kinase SYK (EC 2.7.1.112) (Spleen tyrosine kinase).		
264	ROR1_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR1 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 1).	785	RYPNYMFPSQGITPQ
265	ROR2_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR2 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 2).	230	SFCHFVFPLCDARSR
266	UBPQ_MOUSE	Ubiquitin carboxyl-terminal hydrolase 26 (EC 3.1.2.15) (Ubiquitin thiolesterase 26) (Ubiquitin-specific processing protease 26) (Deubiquitinating enzyme 26).	223	SKPGFGFPFETNYPE
267	UBP8_HUMAN	Ubiquitin carboxyl-terminal hydrolase 8 (EC 3.1.2.15) (Ubiquitin thiolesterase 8) (Ubiquitin-specific processing protease 8) (Deubiquitinating enzyme 8).	330	ISLDFTYPSLEESIP
			537	DSDYFKYPLMALGEL
268	UB4B_HUMAN	Ubiquitin conjugation factor E4 B (Ubiquitin-fusion degradation protein 2).	80	EDPAFGFPKLEQANK
269	27434480	ubiquitin ligase E3 alpha-II [Homo sapiens]		
270	EDD_HUMAN	Ubiquitin--protein ligase EDD (EC 6.3.2.-) (Hyperplastic discs protein homolog) (hHYD) (Progestin induced protein).	274	DISYFGYPSFRRSSL
			1001	GFQAYDFPAVTTAQY
271	SYV2_HUMAN	Valyl-tRNA synthetase 2 (EC 6.1.1.9) (Valine--tRNA ligase 2) (ValRS 2) (G7a).		
272	PPOV_HUMAN	Vault poly(ADP-ribose) polymerase (EC 2.4.2.30) (VPARP) (193-kDa vault protein) (PARP-related/lalphal-related H5/proline-rich) (PH5P).	921	YKELFSYPKHITSNT
			565	RRTGFSFPTQEPRPQ
273	VINE_HUMAN	Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	352	GRRDFVYPSSTRDPS
274	VINE_HUMAN	Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	577	KSLCFQYPPVYVGKI
275	WRN_HUMAN	Werner syndrome helicase.		
276	WAS2_HUMAN	Wiskott-Aldrich syndrome protein family member 2 (WASP-family protein member 2) (Verprolin homology domain-containing protein 2).	270	PPAEFSYPVDNQRGS
277	WAS2_HUMAN	Wiskott-Aldrich syndrome protein family member 2 (WASP-family protein member 2) (Verprolin homology domain-containing protein 2).	218	KLGPFGYPPTLVYQN
			149	PSGAYVFPPPVANGM
278	WBP2_MOUSE	WW domain binding protein 2 (WBP-2).		

279	ZN44_HUMAN	Zinc finger protein 44 (Zinc finger protein KOX7) (Gonadotropin inducible transcription repressor-2) (GIOT-2).	342 CGKGFDFPGSARIHE
280	HRX_HUMAN	Zinc finger protein HRX (ALL-1) (Trithorax-like protein).	792 LNPTFTFPSHSLTQS
281	Z287_HUMAN	Zinc finger protein ZNF287.	44 NFRNFPYPDLAGPRK

TABLE 3: CARDIOVASCULAR

Accession Code	Target Description	Amino Acid	Target Sequence
1	ACHB_HUMAN	Acetylcholine receptor protein, beta chain precursor.	395 PPSDFLFPKPNRFQP
2	CYA4_HUMAN	Adenylate cyclase, type IV (EC 4.6.1.1) (ATP pyrophosphate-lyase) (Adenylyl cyclase).	679 ITSLFFFPTSSDCPF
3	GAT2_HUMAN	Endothelial transcription factor GATA-2.	167 GSHLFGFPPTPPKEV
4	CXA1_HUMAN	Gap junction alpha-1 protein (Connexin 43) (Cx43) (Gap junction 43 kDa heart protein).	330 HAQPFDFPDDNQNSK
5	GBAS_HUMAN	Guanine nucleotide-binding protein G(S), alpha subunit (Adenylate cyclase-stimulating G alpha protein).	136 NVPDFDFPPEFYEHA
6	IKAP_HUMAN	IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150).	599 FPVRFPYPCTQTELA
7	LGR6_HUMAN	Leucine-rich repeat-containing G protein-coupled receptor 6.	751 GLETYGFPSVTLISC
8	MYHD_HUMAN	Myosin heavy chain, skeletal muscle, extraocular (MyHC-eo).	306 STNPFDFPFVSQGEV
9	NDR4_HUMAN	NDRG4 protein (Brain development-related molecule 1) (Vascular smooth muscle cell associated protein-8) (SMAP-8).	80 FPQGYQFPSMEQLAA
10	ACHO_RAT	Neuronal acetylcholine receptor protein, beta-3 chain precursor.	358 PMDRFSFPDGKESDT
11	P2X1_HUMAN	P2X purinoceptor 1 (ATP receptor) (P2X1) (Purinergic receptor).	86 DVADYVFPAQGDNSF
12	P2X7_HUMAN	P2X purinoceptor 7 (ATP receptor) (P2X7) (Purinergic receptor) (P2Z receptor).	89 DTADYTFPLQGNSFF
13	GPR4_HUMAN	Probable G protein-coupled receptor GPR4 (GPR19).	186 VFVGFLFPWALMLLS
14	GP68_HUMAN	Probable G protein-coupled receptor GPR68 (Ovarian cancer G protein- coupled receptor 1) (OGR-1).	190 FLVGFLFPICLLLAS
15	GP17_HUMAN	Probable P2Y purinoceptor GPR17 (P2Y-like receptor) (R12).	225 LAVAFTFPFITTVTC
16	GP40_HUMAN	Putative G protein-coupled receptor GPR40.	244 NVASFLYPNLGGSWR
17	FK79_HUMAN	Putative P2Y purinoceptor FKSG79.	244 APYHFSFPLDFLVKS
18	LGR7_HUMAN	Relaxin receptor 1 (Leucine-rich repeat-containing G protein-coupled receptor 7).	734 KPDLFTYPCEMSLIS
19	AG2R_HUMAN	Type-1 angiotensin II receptor (AT1) (AT1AR).	200 NILGFLFPFLIILTS
20	VNN2_HUMAN	Vascular non-inflammatory molecule 2 precursor (Vanin 2) (Glycosylphosphatidyl inositol-anchored protein GPI-80) (FOAP-4 protein).	435 FGTEYVFPEVLLTEI
21	16904210	very large G protein-coupled receptor 1 [Mus musculus]	1971 PYGVFIFPNKTRPLS
22	VWF_HUMAN	Von Willebrand factor precursor (vWF).	879 DGLKYLFPGECQYVL
23	WS14_HUMAN	Williams-Beuren syndrome chromosome region 14 protein (WS basic-helix- loop-helix leucine zipper protein) (WS-bHLH) (Mlx interactor).	431 FSPRFPFPTVPPAPG

TABLE 4: INFLAMMATION

Acc ssion Code	Target Description	Amino Acid	Target Sequence
	Adaptor-related protein complex 1, mu 2 subunit (Mu-adaptin 2) (Adaptor protein complex AP-1 mu-2 subunit) (Golgi adaptor HA1/AP1 adaptin mu-2 subunit) (Clathrin assembly protein assembly	116	ELMDFGFPQTTDSKI
1 A1M2_HUMAN	protein complex 1 medium chain 2) (AP-mu chain family member mu1B).	97	SGETFSYPDFLRMML
2 AIF1_HUMAN	Allograft inflammatory factor-1 (AIF-1) (Ionized calcium-binding adapter molecule 1) (G1).	23	QVVTFTFPFGFQGIS
3 APL3_HUMAN	Apolipoprotein L3 (Apolipoprotein L-III) (ApoL-III) (TNF-inducible protein CG12-1) (CG12_1).	947	FYCLYSFPSKKSKAR
4 CABI_HUMAN	Calcineurin-binding protein Cabin 1 (Calcineurin inhibitor) (CAIN).	102	GGGDFDYPGAPAGPG
5 CEBA_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	84	DPRPFAYPSHTFGPD
6 CEBE_RAT	CCAAT/enhancer binding protein epsilon (C/EBP epsilon) (C/EBP-related protein 1).	915	ARGSFEFPVGDAVSK
7 CO4_HUMAN	Complement C4 precursor [Contains: C4A anaphylatoxin].	201	THCQYNFPQVGRTAL
8 CCR3_HUMAN	C-X-C chemokine receptor type 3 (CXC-R3) (CXCR-3) (CKR-L2) (CD183 antigen).	167	GSHLFGFPPTPPKEV
9 GAT2_HUMAN	Endothelial transcription factor GATA-2.		
	FYN-binding protein (FYN-T-binding protein) (FYB-120/130) (p120/p130) (SLP-76 associated	125	SKPTFPWPPGNKPSL
10 FYB_HUMAN	phosphoprotein) (SLAP-130).	88	KKDKFAFPVPYGLGS
11 28274770	HMG-box protein SOX21 [Mus musculus]	599	FPVRFPYPCTQTELA
12 IKAP_HUMAN	IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150).		
	Interferon regulatory factor 4 (IRF-4) (Lymphocyte specific interferon regulatory factor) (LSIRF) (NF-	288	DQVLFPYPEDNGQRK
13 IRF4_HUMAN	EM5) (Multiple myeloma oncogene 1).	134	RVCTFRFPSTNIKIT
14 ILF1_HUMAN	Interleukin enhancer-binding factor 1 (Cellular transcription factor ILF-1).	280	QFHSFIYPYMANGSL
15 IRA2_HUMAN	Interleukin-1 receptor-associated kinase-2 (EC 2.7.1.-) (IRAK-2).	249	MMPPFMYPPYLPFPP
16 BAT2_HUMAN	Large proline-rich protein BAT2 (HLA-B-associated transcript 2) (G2).		
	Lipopolysaccharide-responsive and beige-like anchor protein (CDC4-like protein) (Beige-like	203	PDAFFNFPGKSAAAI
17 LRBA_HUMAN	protein).		
	Macrophage metalloelastase precursor (EC 3.4.24.65) (HME) (Matrix metalloproteinase-12) (MMP-	367	SIHSFGFPNFVKKID
18 MM12_HUMAN	12) (Macrophage elastase) (ME).		
	NDRG4 protein (Brain development-related molecule 1) (Vascular smooth muscle cell associated	80	FPQGYQFPSMEQLAA
19 NDR4_HUMAN	protein-8) (SMAP-8).	404	RLPSFNYPSDHLSLV
20 NOCT_HUMAN	Nocturnin (CCR4 protein homolog).		
	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP- 1) [Contains: Nuclear	405	SFPHYGFPTYGGITF
21 KBF1_HUMAN	factor NF-kappa-B p50 subunit].		
	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP- 1) [Contains: Nuclear	400	TGPGYSFPHYGFPTY
22 KBF1_HUMAN	factor NF-kappa-B p50 subunit].		
	Proteinase activated receptor 2 precursor (PAR-2) (Thrombin receptor- like 1) (Coagulation factor II	247	AIGVFLFPAFLTASA
23 PAR2_HUMAN	receptor-like 1).	734	KPDLFTYPCEMSLIS
24 LGR7_HUMAN	Relaxin receptor 1 (Leucine-rich repeat-containing G protein-coupled receptor 7).	22	PPYAFFFPPMLGGLS
25 RRA_HUMAN	Retinoic acid receptor alpha (RAR-alpha).	664	SYLIYVFPDRPKDEV
26 ST5A_HUMAN	Signal transducer and activator of transcription 5A.	664	NYLIYVFPDRPKDEV
27 ST5B_HUMAN	Signal transducer and activator of transcription 5B.	281	GTSHFEFPDYCTPEV
28 SX12_HUMAN	SOX-12 protein (SOX-22 protein).		

29	15277232	TNFalpha-inducible ATP-binding protein [Homo sapiens]	570	YTVRFTFPDPPPLSP
30	TLR1_HUMAN	Toll-like receptor 1 precursor (Toll/interleukin-1 receptor-like) (TIL).	308	VSDVFGFPQSYIYEI
31	TLR4_HUMAN	Toll-like receptor 4 precursor (hToll).	71	SYSFFSFPELQVLDL
32	GAT3_HUMAN	Trans-acting T-cell specific transcription factor GATA-3.	147	SPHLFTFPPTPPKDV
33	TF65_MOUSE	Transcription factor p65 (Nuclear factor NF-kappa-B p65 subunit).	345	APQPYTFPASLSTIN
34	SX11_HUMAN	Transcription factor SOX-11.	407	LGSHFEFPDYCTPEL
35	SX21_HUMAN	Transcription factor SOX-21.	88	KKDKFAFPVPYGLGG
36	SOX4_HUMAN	Transcription factor SOX-4.	440	SGSHFEFPDYCTPEV
37	SOX6_HUMAN	Transcription factor SOX-6.	285	AQQGFLFPPGITYKP
38	SSRA_HUMAN	Translocon-associated protein, alpha subunit precursor (TRAP-alpha) (Signal sequence receptor alpha subunit) (SSR-alpha).	120	LDASFRYPQDYQFYI
39	T11B_HUMAN	Tumor necrosis factor receptor superfamily member 11B precursor (Osteoprotegerin) (Osteoclastogenesis inhibitory factor).	351	HSKTYHFPKTVTQSL
40	T13X_HUMAN	Tumor necrosis factor receptor superfamily member 13B (Transmembrane activator and CAML interactor).	228	ETCSFCFPECRAPTQ
41	AG2R_HUMAN	Type-1 angiotensin II receptor (AT1) (AT1AR).	200	NILGFLFPFLIILTS
42	JAK2_HUMAN	Tyrosine-protein kinase JAK2 (EC 2.7.1.112) (Janus kinase 2) (JAK-2).	114	YRIRFYFPRWYCSGS
43	JAK3_HUMAN	Tyrosine-protein kinase JAK3 (EC 2.7.1.112) (Janus kinase 3) (JAK-3) (Leukocyte janus kinase) (L-JAK).	100	YRIRFYFPNWFGLEK
44	VNN3_HUMAN	Vascular non-inflammatory molecule 3 precursor (Vanin 3).	185	PEIQFDFPKDSELVT
45	VNN3_HUMAN	Vascular non-inflammatory molecule 3 precursor (Vanin 3).	436	FGTRYVFPQIILSGS
46	VINE_HUMAN	Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	565	RRTGFSFPTQEPRPQ
47	VINE_HUMAN	Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	352	GRRDFVYPSSTRDPS
48	ZEP1_HUMAN	Zinc finger protein 40 (Human immunodeficiency virus type I enhancer- binding protein 1) (HIV-EP1) (Major histocompatibility complex binding protein 1) (MBP-1) (Positive regulatory domain II binding factor 1) (PRDII-BF1).	1255	KSEKFSWPQRSETLS

TABLE 5: METABOLIC DISORDERS

Accession Code	Target Description	Amino Acid	Target Sequence
1	PIP3_HUMAN	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta 3 (EC 3.1.4.11) (Phosphoinositide phospholipase C) (PLC-beta-3) (Phospholipase C-beta-3).	777	DEEPFDFPKVVLPTL
2	AAK1_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-1 chain (EC 2.7.1.-) (AMPK alpha-1 chain).	273	DLPKYLFPEDPSYSS
3	AAK2_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-2 chain (EC 2.7.1.-) (AMPK alpha-2 chain).	271	DLPSYLFPEDPSYDA
4	ACDV_HUMAN	Acyl-CoA dehydrogenase, very-long-chain specific, mitochondrial precursor (EC 1.3.99.-) (VLCAD).	84	TDQVFPYPSVLNEEQ
5	ANDR_HUMAN	Androgen receptor (Dihydrotestosterone receptor).	359	SRDYYNFPLALAGPP
6	ANDR_HUMAN	Androgen receptor (Dihydrotestosterone receptor).	547	LPIDYYFPPQKTCLI
7	APB_HUMAN	Apolipoprotein B-100 precursor (Apo B-100) [Contains: Apolipoprotein B-48 (Apo B-48)].	4198	NFPRFQFPGKPGIYT

8	APL3_HUMAN	Apolipoprotein L3 (Apolipoprotein L-III) (ApoL-III) (TNF-inducible protein CG12-1) (CG12_1).	23	QVVTFTFPFGFQGIS
9	AQP1_HUMAN	Aquaporin-CHIP (Water channel protein for red blood cells and kidney proximal tubule) (Aquaporin 1) (AQP-1) (Urine water channel).	31	SALGFKYPVGNNQTA
10	ABC1_HUMAN	ATP-binding cassette, sub-family A, member 1 (ATP-binding cassette transporter 1) (ATP-binding cassette 1) (ABC-1) (Cholesterol efflux regulatory protein).	78	NNPCFRYPTPGEAPG
11	ABF2_HUMAN	ATP-binding cassette, sub-family F, member 2 (Iron inhibited ABC transporter 2) (HUSSY-18).	379	KTLSFYFPPCGKIPP
12	HEXA_HUMAN	Beta-hexosaminidase alpha chain precursor (EC 3.2.1.52) (N-acetyl- beta-glucosaminidase) (Beta-N-acetylhexosaminidase) (Hexosaminidase A).	212	PYESFTFPELMRKGS
13	AB11_HUMAN	Bile salt export pump (ATP-binding cassette, sub-family B, member 11).	1081	VDCKFTYPSRPDSQV
14	BIEA_HUMAN	Biliverdin reductase A precursor (EC 1.3.1.24) (Biliverdin-IX alpha- reductase).	158	EEERFGFPAFSGISR
15	CEBA_BOVIN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	27	SSAAFGFPRGAGPSQ
16	CEBA_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	27	SSAAFGFPRGAGPAQ
17	CEBE_HUMAN	CCAAT/enhancer binding protein epsilon (C/EBP epsilon).	84	DPRPFAYPPHTFGPD
18	13562153	channel-kinase 1 [Homo sapiens]	1339	QRKEFNFPEAGSSSG
19	CLC3_HUMAN	Chloride channel protein 3 (CIC-3).	236	NIFSYLFPKYSTNEA
20	CLC3_HUMAN	Chloride channel protein 3 (CIC-3).	280	EEVSYYFPLKTLWRS
21	CLC6_HUMAN	Chloride channel protein 6 (CIC-6).	223	RKIQFNFPYFRSDRD
22	CICL_HUMAN	Chloride channel protein CLC-KB (CIC-K2).	442	ETLSFIFPEGIVAGG
23	CLI6_HUMAN	Chloride intracellular channel 6.	638	KYRDFEFPSEMTGIW
24	CETP_HUMAN	Cholesteryl ester transfer protein precursor (Lipid transfer protein I).	474	LQMDFGFPEHLLVDF
25	CNG4_HUMAN	Cyclic-nucleotide-gated cation channel 4 (CNG channel 4) (CNG-4) (CNG4) (Cyclic nucleotide-gated cation channel modulatory subunit).	296	PWKKYQFPQSIDPLT
26	C561_HUMAN	Cytochrome b561 (Cytochrome b-561).	139	GFSFFLFPGASFSLR
27	CX42_HUMAN	Cytochrome c oxidase subunit IV isoform 2, mitochondrial precursor (EC 1.9.3.1) (COX IV-2).	122	WQRVYVFPPKPITLT
28	CP27_HUMAN	Cytochrome P450 27, mitochondrial precursor (EC 1.14.-.-) (Cytochrome P-450C27/25) (Sterol 26-hydroxylase) (Sterol 27-hydroxylase) (Vitamin D(3) 25-hydroxylase) (5-beta-cholestane-3-alpha,7-alpha,12-alpha-triol 27-hydroxylase).	412	EVDGFLFPKNTQFVF
29	12832024	dJ686C3.1.3 (isocitrate dehydrogenase 3 (NAD+) beta (isoform C)) [Homo sapiens]	7	WSSLFPFPVSPSCCF
30	ENP4_HUMAN	Ectonucleoside triphosphate diphosphohydrolase 4 (EC 3.6.1.6) (NTPDase4) (Uridine-diphosphatase) (UDPase) (Lysosomal apyrase-like protein of 70 kDa).	500	FHRGFSFPVNYKSLK
31	EAA1_HUMAN	Excitatory amino acid transporter 1 (Sodium-dependent glutamate/aspartate transporter 1) (Glial glutamate transporter) (GLAST-1).	77	EVKYFSFPGELLMRM
32	EAA4_HUMAN	Excitatory amino acid transporter 4 (Sodium-dependent glutamate/aspartate transporter).	85	QIKYFSFPGELLMRM
33	EAA5_HUMAN	Excitatory amino acid transporter 5 (Retinal glutamate transporter).	46	EISYFQFPGELLMRM
34	F16P_HUMAN	Fructose-1,6-bisphosphatase (EC 3.1.3.11) (D-fructose-1,6-bisphosphate 1-phosphohydrolase) (FBPase).	258	YGGIFLYPANKKSPN
35	F16Q_HUMAN	Fructose-1,6-bisphosphatase isozyme 2 (EC 3.1.3.11) (D-fructose-1,6- bisphosphate 1-phosphohydrolase) (FBPase).	259	YGGIFLYPANQKSPK

		Galactosylceramide sulfotransferase (EC 2.8.2.11) (GalCer sulfotransferase) (Cerebroside sulfotransferase) (3'- phosphoadenylylsulfate:galactosylceramide 3'-sulfotransferase) (3'-	102 HRLKFAFPNGRNDFD
36	CST_HUMAN	phosphoadenosine-5'phosphosulfate:GalCer sulfotransferase).	162 EPENFSFPDDLQCVD
37	KLK1_HUMAN	Glandular kallikrein 1 precursor (EC 3.4.21.35) (Tissue kallikrein) (Kidney/pancreas/salivary gland kallikrein).	131 KVIDFHYPNELLQEY
38	DCE2_HUMAN	Glutamate decarboxylase, 65 kDa isoform (EC 4.1.1.15) (GAD-65) (65 kDa glutamic acid decarboxylase).	125 DHADYGFPSCEGKFN
39	GSHR_HUMAN	Glutathione reductase, mitochondrial precursor (EC 1.8.1.7) (GR) (GRase).	260 SGVLFQYPDTEGKVE
40	GCSP_HUMAN	Glycine dehydrogenase [decarboxylating], mitochondrial precursor (EC 1.4.4.2) (Glycine decarboxylase) (Glycine cleavage system P- protein).	
		Glycogen debranching enzyme (Glycogen debrancher) [Includes: 4-alpha- glucanotransferase (EC 2.4.1.25) (Oligo-1,4-1,4-glucantransferase); Amylo-alpha-1,6-glucosidase (EC 3.2.1.33) (Amylo-1,6-	426 VTRYFTFPFEEIDFS
41	GDE_HUMAN	glucosidase) (Dextrin 6-alpha-D-glucosidase)].	350 NYTEFKFPQIKAHPW
42	KG3A_HUMAN	Glycogen synthase kinase-3 alpha (EC 2.7.1.37) (GSK-3 alpha).	601 QRTFFSFPAVVAPFK
43	SYG_HUMAN	Glycyl-tRNA synthetase (EC 6.1.1.14) (Glycine--tRNA ligase) (GlyRS).	
		Guanine deaminase (EC 3.5.4.3) (Guanase) (Guanine aminase) (Guanine aminohydrolase) (GAH)	102 WLTKYTFPAEHRFQN
44	GUAD_HUMAN	(p51-nedasin).	163 GLAFFTFPNIASATK
45	HO1_HUMAN	Heme oxygenase 1 (EC 1.14.99.3) (HO-1).	
		Hepatocyte nuclear factor 1-beta (HNF-1B) (Variant hepatic nuclear factor 1) (VHNF1)	200 DQLLFLFPEFSQQSH
46	HNFB_HUMAN	(Homeoprotein LFB3) (Transcription factor 2) (TCF-2).	146 LGFTFSFPVRHEDID
47	HXK4_HUMAN	Hexokinase D (EC 2.7.1.1) (Hexokinase type IV) (HK IV) (HK4) (Glucokinase).	163 LGFSFSFPCHQTGLD
48	HXK3_HUMAN	Hexokinase type III (EC 2.7.1.1) (HK III).	598 LGFTFSFPCQQTSLD
49	HXK1_HUMAN	Hexokinase, type I (EC 2.7.1.1) (HK I) (Brain form hexokinase).	598 LGFTFSFPCQQNSLD
50	HXK2_HUMAN	Hexokinase, type II (EC 2.7.1.1) (HK II) (Muscle form hexokinase).	599 FPVRFPYPCTQTELA
51	IKAP_HUMAN	IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150).	280 QFHSFIYPYMANGSL
52	IRA2_HUMAN	Interleukin-1 receptor-associated kinase-2 (EC 2.7.1.-) (IRAK-2).	78 VVSAFGFPVILARVA
53	OBRG_HUMAN	Leptin receptor gene-related protein (OB-R gene related protein) (OB- RGRP).	142 MEEEFNYPLDNVHLL
54	LIPL_HUMAN	Lipoprotein lipase precursor (EC 3.1.1.34) (LPL).	
55	LRP2_HUMAN	Low-density lipoprotein receptor-related protein 2 precursor (Megalin) (Glycoprotein 330) (gp330).	1025 QCGLFSFPCKNGRCV
56	LOL1_HUMAN	Lysyl oxidase homolog 1 precursor (EC 1.4.3.-) (Lysyl oxidase-like protein 1) (LOL).	173 YPQQFPYPQAPFVSQ
57	MDHC_HUMAN	Malate dehydrogenase, cytoplasmic (EC 1.1.1.37).	281 DDLLYSFPVVIKNKT
58	MUTA_HUMAN	Methylmalonyl-CoA mutase, mitochondrial precursor (EC 5.4.99.2) (MCM).	227 VRNTYIFPPEPSMKI
		Mitochondrial 28S ribosomal protein S29 (S29mt) (MRP-S29) (Death- associated protein 3) (DAP-3)	113 KNTSFAYPAIRYLLY
59	RT29_HUMAN	(Ionizing radiation resistance conferring protein).	2 HGQTFTFPDLFPEKD
60	14141575	mitochondrial ribosomal protein L23 [Homo sapiens]	30 IGFSYAFPKSITVFF
61	MOT1_HUMAN	Monocarboxylate transporter 1 (MCT 1).	30 IGFSYAFPKAVTVFF
62	MOT2_HUMAN	Monocarboxylate transporter 2 (MCT 2).	29 TGFAYGFPKAVSVFF
63	MOT3_HUMAN	Monocarboxylate transporter 3 (MCT 3).	32 TGFSYAFPKAVSVFF
64	MOT4_HUMAN	Monocarboxylate transporter 4 (MCT 4) (MCT 3).	

65	MYHD_HUMAN	Myosin heavy chain, skeletal muscle, extraocular (MyHC-eo).	306	STNPFDFPFVSQGEV
66	PIGA_HUMAN	N-acetylglucosaminyl-phosphatidylinositol biosynthetic protein (GlcNAc-PI synthesis protein) (Phosphatidylinositol-glycan biosynthesis, class A protein) (PIG-A).	37	MVSDFFYPNMGGVES
67	NB8M_HUMAN	NADH-ubiquinone oxidoreductase B18 subunit (EC 1.6.5.3) (EC 1.6.99.3) (Complex I-B18) (CI-B18) (Cell adhesion protein SQM1).	24	FPPDYGFPERKEREM
68	NI2M_HUMAN	NADH-ubiquinone oxidoreductase B22 subunit (EC 1.6.5.3) (EC 1.6.99.3) (Complex I-B22) (CI-B22).	75	HPQPYIFPDSPGGTS
69	MAOX_HUMAN	NADP-dependent malic enzyme (EC 1.1.1.40) (NADP-ME) (Malic enzyme 1).	452	GNNSYVFPGVALGVV
70	NPH4_HUMAN	Nephrocystin 4 (Nephroretinin).	669	TFQFYRFPPATTPRL
71	NCO2_HUMAN	Nuclear receptor coactivator 2 (NCoA-2) (Transcriptional intermediary factor 2).	1295	NAQQFPFPPNYGISQ
72	NCR1_HUMAN	Nuclear receptor co-repressor 1 (N-CoR1) (N-CoR).	23	HSVQYTFPNTRHQQE
73	NCR2_HUMAN	Nuclear receptor co-repressor 2 (N-CoR2) (Silencing mediator of retinoic acid and thyroid hormone receptor) (SMRT) (SMRTe) (Thyroid-, retinoic-acid-receptor-associated co-repressor) (T3 receptor-associating factor) (TRAC) (CTG repeat protein 26).	2160	PAPLYSFPGASCPVL
74	RORG_HUMAN	Nuclear receptor ROR-gamma (Nuclear receptor RZR-gamma).	540	PPSPFSFPMNPGGWS
75	STT3_HUMAN	Oligosaccharyl transferase STT3 subunit homolog (B5) (Integral membrane protein 1) (TMC).	365	QLLVFMFPVGLYYCF
76	PNK4_HUMAN	Pantothenate kinase 4 (EC 2.7.1.33) (Pantothenic acid kinase 4) (hPanK4).	513	CLNEFNFPDPYSKVK
77	PPAR_HUMAN	Peroxisome proliferator activated receptor alpha (PPAR-alpha).	417	PDDIFLFPKLLQKMA
78	PPAS_MOUSE	Peroxisome proliferator activated receptor delta (PPAR-delta) (PPAR-beta) (Nuclear hormone receptor 1) (NUC1).	389	PDSQYLFPKLLQKMA
79	P11D_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, delta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit delta) (PtdIns- 3-kinase p110) (PI3K) (p110delta).	581	ELLDFSFPDCHVGSF
80	PIGO_HUMAN	Phosphatidylinositol-glycan biosynthesis, class O protein (PIG-O).	190	FSKAFFFPSFNVRDL
81	940231	phosphodiesterase A' subunit [Homo sapiens]	366	ADEYFTFPKGPVDET
82	KPRA_HUMAN	Phosphoribosyl pyrophosphate synthetase-associated protein 1 (PRPP synthetase-associated protein 1) (39 kDa phosphoribosypyrophosphate synthetase-associated protein) (PAP39).	140	IQGFFSFPVDNLRAS
83	PIT1_HUMAN	Pituitary-specific positive transcription factor 1 (Pit-1) (Growth hormone factor 1) (GHF-1).	75	TPCLYKFPDHTLSHG
84	PKHD_HUMAN	Polycystic kidney and hepatic disease 1 precursor (Fibrocystin) (Polyductin) (Tigmin).	3321	DKNKFYFPSLQPRKD
85	PKHD_HUMAN	Polycystic kidney and hepatic disease 1 precursor (Fibrocystin) (Polyductin) (Tigmin).	2512	NLVAFPFPHAAILED
86	PKHD_HUMAN	Polycystic kidney and hepatic disease 1 precursor (Fibrocystin) (Polyductin) (Tigmin).	2370	RYGLFVYPKFQPPWD
87	P1L1_HUMAN	Polycystic kidney disease 1-like 1 protein (Polycystin 1L1).	2687	PGLLFHFPRRSQKDC
88	PKDR_HUMAN	Polycystic kidney disease and receptor for egg jelly related protein precursor (PKD and REJ homolog).	1880	GYALYFFPEQQRFNS
89	PKD1_HUMAN	Polycystin 1 precursor (Autosomal dominant polycystic kidney disease protein 1).	3060	SHVRFVFPEPTADVN
90	PAGT_HUMAN	Polypeptide N-acetylgalactosaminyltransferase (EC 2.4.1.41) (Protein- UDP acetylgalactosaminyltransferase) (UDP-GalNAc:polypeptide, N- acetylgalactosaminyltransferase) (GalNAc-T1).	348	KATPYTFPGGTGQII
91	DP1_HUMAN	Polyposis locus protein 1 (TB2 protein).	56	NLIGFGYPAYISIKA

92	CIK1_HUMAN	Potassium voltage-gated channel subfamily A member 1 (Potassium channel Kv1.1) (HUKI) (HBK1).	153	VWLLFEYPESSGPAR
93	CIK5_HUMAN	Potassium voltage-gated channel subfamily A member 5 (Potassium channel Kv1.5) (HK2) (HPCN1).	236	VWLIFEYPESSGSAR
94	KCH7_HUMAN	Potassium voltage-gated channel subfamily H member 7 (Ether-a-go-go related gene potassium channel 3) (HERG-3) (Ether-a-go-go related protein 3) (Eag related protein 3).	156	KFFGFKFPGLRVLTY
			942	ETNVFFYPRLLPLTK
95	S24C_HUMAN	Protein transport protein Sec24C (SEC24-related protein C).	370	GNTPFIFPLYGHGEI
96	6007826	rab escort protein-2 [Mus musculus]		
97	NPT2_HUMAN	Renal sodium-dependent phosphate transport protein 2 (Sodium/phosphate cotransporter 2) (Na(+)/Pi cotransporter 2) (Renal sodium-phosphate transport protein 2) (Renal Na(+)-dependent phosphate cotransporter 2).	43	GTSAYAFPSLGPVAL
			22	PPYAFFFPPMLGGLS
98	RRA_HUMAN	Retinoic acid receptor alpha (RAR-alpha).	23	AGFPFAFPGALRGSP
99	RRG1_HUMAN	Retinoic acid receptor gamma-1 (RAR-gamma-1).	370	PSQPYMFPRMLMKIT
100	RRG1_HUMAN	Retinoic acid receptor gamma-1 (RAR-gamma-1).	1830	PISLFSFPPLLPQQF
101	12053793	retinoid-acid induced protein 1 [Homo sapiens]		
102	SIL5_HUMAN	Sialic acid binding Ig-like lectin 5 precursor (Siglec-5) (Obesity- binding protein 2) (OB binding protein-2) (OB-BP2) (CD33 antigen-like 2) (CD170 antigen).	39	VPCSFSYPWRSWYSS
			120	GGGAFMFPYFIMLIF
103	S6A9_HUMAN	Sodium- and chloride-dependent glycine transporter 1 (GlyT1) (GlyT-1).		
104	NTCP_HUMAN	Sodium/bile acid cotransporter (Na(+)/bile acid cotransporter) (Na(+)/taurocholate transport protein) (Sodium/taurocholate cotransporting polypeptide).	279	IGPLFFFPLLYMIFQ
			835	PRSSFAFPPSLAKAG
105	NAH5_HUMAN	Sodium/hydrogen exchanger 5 (Na(+)/H(+) exchanger 5) (NHE-5).	525	EYGSYRFPPWAELLG
106	S6A7_HUMAN	Sodium-dependent proline transporter.		
107	S21B_HUMAN	Solute carrier family 21 member 11 (Sodium-independent organic anion transporter D) (Organic anion transporting polypeptide D) (OATP-D) (Organic anion transporter polypeptide related protein 3) (OATP-RP3) (OATPRP3) (PGE1 transporter).	279	SLLMFGFPQSLPPHS
			296	FDELFQFPVVYDQLS
108	SYTA_MOUSE	Synaptotagmin X (SytX).	163	FSVDYNFPKKALVVT
109	SYTB_HUMAN	Synaptotagmin XI (SytXI).		
110	T240_HUMAN	Thyroid hormone receptor-associated protein complex 240 kDa component (Trap240) (Activator-recruited cofactor 250 kDa component) (ARC250).	703	GDEEFLFPDKKDRQN
111	T240_HUMAN	Thyroid hormone receptor-associated protein complex 240 kDa component (Trap240) (Activator-recruited cofactor 250 kDa component) (ARC250).	614	AWKYYKFPKKKDVEF
			1536	KTCPFFFPFDTRQML
112	TRIB_HUMAN	Thyroid receptor interacting protein 12 (TRIP12).	570	YTVRFTFPDPPPLSP
113	15277232	TNFalpha-inducible ATP-binding protein [Homo sapiens]		
114	TERA_HUMAN	Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)- ATPase p97 subunit) (Valosin containing protein) (VCP) [Contains: Valosin].	767	GFGSFRFPSGNQGGA
			498	FTMDFCFPFSPLQAF
115	TUL2_HUMAN	Tubby related protein 2 (Tubby-like protein 2).	1518	YILDFQYPFSAVQAF
116	TUSP_HUMAN	Tubby superfamily protein.		
117	T11B_HUMAN	Tumor necrosis factor receptor superfamily member 11B precursor (Osteoprotegerin) (Osteoclastogenesis inhibitory factor).	351	HSKTYHFPKTVTQSL

		Tumor necrosis factor receptor superfamily member 13B (Transmembrane activator and CAML	228	ETCSFCFPECRAPTQ
118	T13X_HUMAN	interactor).	114	YRIRFYFPRWYCSGS
119	JAK2_HUMAN	Tyrosine-protein kinase JAK2 (EC 2.7.1.112) (Janus kinase 2) (JAK-2).		
		UDP-glucuronosyltransferase 2B15 precursor, microsomal (EC 2.4.1.17) (UDPGT) (UDPGTH-3)	184	NGGGFLFPPSYVPVV
120	UDBF_HUMAN	(HLUG4).		
		Vasopressin V1b receptor (V1bR) (AVPR V1b) (Vasopressin V3 receptor) (AVPR V3) (Antidiuretic	186	CWADFGFPWGPRAYL
121	V1BR_HUMAN	hormone receptor 1b).	1971	PYGVFIFPNKTRPLS
122	16904210	very large G protein-coupled receptor 1 [Mus musculus]	577	KSLCFQYPPVYVGKI
123	WRN_HUMAN	Werner syndrome helicase.	413	FFVIFSFPIASKDCI
124	WFS1_HUMAN	Wolframin.		
		Zinc finger protein 44 (Zinc finger protein KOX7) (Gonadotropin inducible transcription repressor-2)	342	CGKGFDFPGSARIHE
125	ZN44_HUMAN	(GIOT-2).		

TABLE 6: NEUROPATHIES

Acc ssion Cod	Target Description	Amino Acid	Target Sequence
	5-hydroxytryptamine 1B receptor (5-HT-1B) (Serotonin receptor) (5-HT-1D-beta) (Serotonin 1D beta	213	TVGAFYFPTLLLIAL
1 5H1B_HUMAN	receptor) (S12).	42	DGGRFKFPDGVQNWP
2 5H2C_HUMAN	5-hydroxytryptamine 2C receptor (5-HT-2C) (Serotonin receptor) (5HT-1C).		
	5-hydroxytryptamine 3 receptor precursor (5-HT-3) (Serotonin-gated ion channel receptor) (5-	158	SLDIYNFPFDVQNCS
3 5HT3_HUMAN	HT3R).	1057	GGERFPYPSFHWDPI
4 AUT2_HUMAN	Autism susceptibility gene 2 protein.	649	CAHWFQYPFDPSFDD
5 BIR1_HUMAN	Baculoviral IAP repeat-containing protein 1 (Neuronal apoptosis inhibitory protein).	318	GVRSFGFPRPQQAAY
6 BRA1_HUMAN	Brain link protein-1 precursor.	341	ELQQFGFPAPQTGDP
7 BAI1_HUMAN	Brain-specific angiogenesis inhibitor 1 precursor.	241	SYAKFLYPTNALVTH
8 CBL2_HUMAN	Cdk5 and abl enzyme substrate 2 (Interactor with cdk3 2) (Ik3-2).	2649	TVFAFNFPSLDALNT
9 DYHB_HUMAN	Ciliary dynein heavy chain 11 (Axonemal beta dynein heavy chain 11).		
	Dimethylaniline monooxygenase [N-oxide forming] 5 (EC 1.14.13.8) (Hepatic flavin-containing	329	TGYSFDFPFLEDSVK
10 FMO5_HUMAN	monooxygenase 5) (FMO 5) (Dimethylaniline oxidase 5).	61	VGTLFRFPGGVSGEE
11 DIS1_HUMAN	Disrupted in schizophrenia 1 protein.		
		309	GAKAFYYPKEAGVPF
12 DOPO_MOUSE	Dopamine beta-monooxygenase precursor (EC 1.14.17.1) (Dopamine beta- hydroxylase) (DBH).	80	TLQIFPFPPSSFSTL
13 DSCA_HUMAN	Down syndrome cell adhesion molecule precursor (CHD2).	598	FIQPFEFPRFSIGQR
14 DSCA_HUMAN	Down syndrome cell adhesion molecule precursor (CHD2).	89	TEIPFEFPLHLKGNK
15 DSR3_HUMAN	Down syndrome critical region protein 3 (Down syndrome critical region protein A).	1867	FNWRFIFPFDYLPAE
16 DYSF_HUMAN	Dysferlin (Dystrophy associated fer-1-like protein) (Fer-1 like protein 1).		
	Ephrin type-A receptor 7 precursor (EC 2.7.1.112) (Tyrosine-protein kinase receptor EHK-3) (Eph	596	LYFHFKFPGTKTYID
17 EPA7_HUMAN	homology kinase-3) (Receptor protein- tyrosine kinase HEK11).	687	SLFDFIFPGKLGTLP
18 ERC6_HUMAN	Excision repair protein ERCC-6 (Cockayne syndrome protein CSB).		

19	EAA3_HUMAN	Excitatory amino acid transporter 3 (Sodium-dependent glutamate/aspartate transporter 3) (Excitatory amino-acid carrier 1) (Neuronal and epithelial glutamate transporter).	48	EKFYFAFPGEILMRM
20	FXGA_HUMAN	Forkhead box protein G1A (Forkhead-related protein FKHL2) (Transcription factor BF-2) (Brain factor 2) (BF2) (HFK2).	391	GQTSYFFPHVPHPSM
21	FMR2_HUMAN	Fragile X mental retardation 2 protein (Protein FMR-2) (FMR2P) (Ox19 protein) (Fragile X E mental retardation syndrome protein).	639	STDEFTWPKPNITSS
22	FMR2_HUMAN	Fragile X mental retardation 2 protein (Protein FMR-2) (FMR2P) (Ox19 protein) (Fragile X E mental retardation syndrome protein).	193	LEDFFVYPAEQPQIG
23	FCMD_HUMAN	Fukutin precursor (Fukuyama-type congenital muscular dystrophy protein).	388	KKFKYLFPKFTLCWT
24	GAE_HUMAN	Gamma-aminobutyric-acid receptor epsilon subunit precursor (GABA(A) receptor).	212	SFSSFSYPENEMIYK
25	GAAT_HUMAN	Gamma-aminobutyric-acid receptor theta subunit precursor (GABA(A) receptor).	609	KWSRFLFPLAFGLFN
26	SGCG_HUMAN	Gamma-sarcoglycan (Gamma-SG) (35 kDa dystrophin-associated glycoprotein) (35DAG).	81	GESEFLFPLYAKEIH
27	KG3A_HUMAN	Glycogen synthase kinase-3 alpha (EC 2.7.1.37) (GSK-3 alpha).	350	NYTEFKFPQIKAHPW
28	17384611	kainate receptor subunit KA2a [Homo sapiens]	945	DASSFFFPPISSCPP
29	OPRK_HUMAN	Kappa-type opioid receptor (KOR-1).	340	CFRDFCFPLKMRMER
30	13383464	kinesin-related protein HASH [Mus musculus]	46	NTRDFMFPGPNQMSG
31	MGR1_HUMAN	Metabotropic glutamate receptor 1 precursor (mGluR1).	3	GLLLFFFPAIFLEVS
32	9988950	monooxygenase X [Homo sapiens]	9	RYPDFSFPYFPQDYF
33	MY15_HUMAN	Myosin XV (Unconventional myosin-15).	751	RGAAFGFPGASPRAS
34	MTR2_HUMAN	Myotubularin-related protein 2.	178	NLMKYAFPVSNNLPL
35	2736151	mytonic dystrophy kinase-related Cdc42-binding kinase [Rattus norvegicus]	297	HKERFQFPTQVTDVS
36	NCA2_HUMAN	Neural cell adhesion molecule 1, 120 kDa isoform precursor (N-CAM 120) (NCAM-120) (CD56 antigen).	425	TCEVFAYPSATISWF
37	NTC3_HUMAN	Neurogenic locus notch homolog protein 3 precursor (Notch 3).	1618	ERLDFPYPLRDVRGE
38	NGN1_HUMAN	Neurogenin 1 (Neurogenic differentiation factor 3) (NeuroD3) (Neurogenic basic-helix-loop-helix protein).	213	GDPVFSFPSLPKDLL
39	NTR1_HUMAN	Neurotensin receptor type 1 (NT-R-1) (High-affinity levocabastine- insensitive neurotensin receptor) (NTRH).	241	TFMSFIFPMVVISVL
40	AAAT_HUMAN	Neutral amino acid transporter B(0) (ATB(0)) (Sodium-dependent neutral amino acid transporter type 2) (RD114/simian type D retrovirus receptor) (Baboon M7 virus receptor).	85	RLSAFVFPGELLLRL
41	OAA3_HUMAN	Olfactory receptor 10A3 (HTPCRX12).	160	TTWVFSFPFCGPNEI
42	OAA5_HUMAN	Olfactory receptor 10A5 (HP3) (Olfactory receptor-like protein JCG6).	161	TTWLFSFPFCGTNKV
43	OXB2_HUMAN	Olfactory receptor 51B2 (HOR5'beta3).	275	SYIYFLFPPLMNPVI
44	OXB4_HUMAN	Olfactory receptor 51B4 (HOR5'beta1).	274	SYVHFLFPPFVNPII
45	OXI1_HUMAN	Olfactory receptor 51I1 (HOR5'beta11).	155	FTTLFPFPFVVKRLP
46	O6B1_HUMAN	Olfactory receptor 6B1 (Olfactory receptor 7-3) (OR7-3).	203	ALVIFLFPLFITVLS
47	PAN1_HUMAN	Pannexin 1.	187	SESHFKYPIVEQYLK
48	RELN_HUMAN	Reelin precursor (EC 3.4.21.-).	1894	LMDEFYFPQTTNILF
49	RELN_HUMAN	Reelin precursor (EC 3.4.21.-).	1967	EDNWFFYPGGNIGLY
50	SACS_HUMAN	Sacsin.	1944	NCTMFRFPLRNAEMA

51	SAD1_HUMAN	SAM domain and HD domain-containing protein 1 (Dendritic cell-derived IFNG-induced protein) (DCIP) (Monocyte protein 5) (MOP-5).	151	GGGYYVFPGASHNRF
52	KPT3_HUMAN	Serine/threonine protein kinase PCTAIRE-3 (EC 2.7.1.-).	371	EFRTYSFPCYLPQPL
53	SAMP_HUMAN	Serum amyloid P-component precursor (SAP) (9.5S alpha-1-glycoprotein).	24	SGKVFVFPRESVTDH
54	SHK2_RAT	SH3 and multiple ankyrin repeat domains protein 2 (Shank2) (Proline- rich synapse associated protein 1) (ProSAP1) (Cortactin-binding protein 1) (CortBP1) (GKAP/SAPAP interacting protein) (SPANK-3).	131	SLSTFEYPGPRRKLY
55	20073220	similar to dJ309K20.4 (KIAA0765, putative brain nuclearly targeted protein (HRIHFB2091, RNA recognition motif (RNP, RRM or RBD domain) containing protein)) [Mus musculus]	631	NGPPFNFPGNFGGPN
56	20071167	Similar to Per1 interacting protein [Mus musculus]	551	PMNPFRFPKEAASLF
57	S6A1_HUMAN	Sodium- and chloride-dependent GABA transporter 1.	523	TMGNYVFPKWGQGVG
58	SX14_HUMAN	Transcription factor SOX-14.	88	KKDRYVFPLPYLGDT
59	ROR1_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR1 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 1).	785	RYPNYMFPSQGITPQ
60	ROR1_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR1 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 1).	226	SLCHYAFPYCDETSS
61	ROR2_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR2 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 2).	230	SFCHFVFPLCDARSR
62	WS14_HUMAN	Williams-Beuren syndrome chromosome region 14 protein (WS basic-helix- loop-helix leucine zipper protein) (WS-bHLH) (Mlx interactor).	431	FSPRFPFPTVPPAPG
63	WFS1_HUMAN	Wolframin.	413	FFVIFSFPIASKDCI

TABLE 7: MISCELLANEOUS DEF DOMAIN-CONTAINING PROTEINS

	Accession Code	Target Description	Amino Acid	Target Sequence
1	15425674	Per1 interacting protein of the suprachiamatic nucleus [Rattus norvegicus]	1023	SLNPFRFPKEAASLF
2	PER1_HUMAN	Period circadian protein 1 (Circadian pacemaker protein Rigui) (hPER).	922	VLPNYLFPTPSSYPY
3	PER2_HUMAN	Period circadian protein 2.	907	MLPSYSFPSGTPNLP
4	PER3_HUMAN	Period circadian protein 3 (hPER3).	843	PYPAFPFPYLDTFMT
5	AK11_HUMAN	A-kinase anchor protein 11 (Protein kinase A anchoring protein 11) (PRKA11) (A kinase anchor protein 220 kDa) (AKAP 220) (hAKAP220).	661	EVCQFSYPQTPASPQ
6	AKA3_HUMAN	A-kinase anchor protein 3 (Protein kinase A anchoring protein 3) (PRKA3) (A-kinase anchor protein 110 kDa) (AKAP 110) (Sperm oocyte binding protein) (Fibrousheathin I) (Fibrous sheath protein of 95 kDa) (FSP95).	490	SDISFEYPEDIGNLS

Claims

1. A method for identifying therapeutic compounds, said method comprising:

(i) providing test cells, wherein said test cells express a target protein containing a DEF domain and a MAP kinase,

(ii) culturing said test cells in the presence of a growth factor, cytokine, tumor promoter, or oncogene,

(iii) contacting said cells with a candidate compound, and

(iv) assessing the binding of said MAP kinase to said target protein, wherein a candidate compound that inhibits said binding is a therapeutic compound.

2. A method for identifying a therapeutic compound, said method comprising:

(i) providing a target protein comprising a DEF domain, a MAP kinase, and a candidate compound,

(ii) mixing said target protein, said MAP kinase, and said candidate compound,

(iii) measuring the binding of said MAP kinase to said target protein, and

(iv) identifying said candidate compound as a therapeutic compound, wherein said candidate compound inhibits said binding, compared to the binding of said MAP kinase to said target protein in the absence of said candidate compound.

3. The method of claim 1 or 2, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

4. The method of claim 3, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

5. The method of claim 4, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

6. The method of claim 1, wherein said test cells are selected from the group consisting of fibroblasts, a primary cell line, an immortalized cell line, and a tumor-derived cell line.

7. The method of claim 1, wherein said test cells are human.

8. The method of claim 1, wherein said growth factor, tcytokine, tumor promoter, or oncogene is selected from the group consisting of epidermal growth factor (EGF), transforming growth factor α, heparin-binding-like EGF, heregulin, amphiregulin, epiregulin, cripto, PDGF-AA, PDGF-BB or PDGF-CC, insulin, insulin-like growth factors, fibroblast growth factors, colony stimulating factor, heaptocyte growth factor, a chemokine, an interleukin, lysophosphatidic acid, a phorbol ester, okadaic acid, microcystin, vanadate, hydrogen peroxide, calyculin A, Erb2/neu, sis, kit, Ras, Raf, PI3-kinase, and PTEN.

9. The method of claim 1 or 2, wherein said MAP kinase is extracellular signal-regulated kinase 1/2 (ERK1/2).

10. The method of claim 1 or 2, wherein said target protein is a Fos, Myc, or Jun family protein.

11. The method of claim 10, wherein said target protein is c-Fos.

12. The method of claim 1 or 2, wherein said therapeutic is useful for the treatment of cancer and said target protein comprises the sequence of a protein identified in Tables 1 or 2.

13. The method of claim 1 or 2, wherein said therapeutic is useful for the

treatment of a cardiovascular disorder and said target protein comprises the sequence of a protein identified in Table 3.

14. The method of claim 1 or 4, wherein said therapeutic is useful for the treatment of an inflammatory disorder, and said target protein comprises the sequence of a protein identified in Table 4.

15. The method of claim 1 or 2, wherein said therapeutic is useful for the treatment of a metabolic disorder, and said target protein comprises the sequence of a protein identified in Table 5.

16. The method of claim 1 or 2, wherein said therapeutic is useful for the treatment of a neuropathy or a behavioral disorder, and said target protein comprises the sequence of a protein identified in Table 6.

17. The method of claim 1 or 2, wherein said therapeutic is useful for the treatment of a sleep disorder, and said target protein comprises the sequence of a protein identified in Table 7.

18. The method of claim 1, wherein said target protein is c-Fos and step (vi) comprises assessing the phosphorylation of T325 or T331.

19. The method of claim 18, wherein said step (vi) comprises an antibody that specifically binds to phospho-T-325 c-Fos.

20. The method of claim 2, wherein said target protein further comprises a fluorescent moiety.

21. The method of claim 20, wherein said fluorescent moiety is fluorescein.

22. A method for treating a cancer in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

23. The method of claim 22, wherein said MAP kinase is ERK1/2.

24. The method of claim 22, wherein said target protein is an immediate early gene.

25. The method of claim 22, wherein said target protein is selected from the group consisting of c-Fos, Fra-1, Fra-2, c-Myc, N-Myc, JunD, JunB, and c-Jun.

26. The method of claim 25, wherein said target protein is c-Fos.

27. The method of claim 22, wherein said target protein is a protein identified in Tables 1 or 2.

28. The method of claim 22, wherein said cancer is selected from the group consisting of leukemia, Hodgkin's disease lymphoma, non-Hodgkin's disease lymphoma, fibrosarcoma, liposarcoma, osteogenic sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

29. The method of claim 22, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

30. The method of claim 22, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

31. The method of claim 22, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

32. A method for treating cardiovascular disease in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

33. The method of claim 32, wherein said MAP kinase is ERK1/2.

34. The method of claim 32, wherein said target protein is a protein identified in Table 3.

35. The method of claim 32, wherein said cardiovascular disease is selected from the group consisting of ischemic heart disease, ventricular heart failure, cardiac hypertrophy, hypertension, and atherosclerosis.

36. The method of claim 32, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

37. The method of claim 32, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

38. The method of claim 32, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

39. A method for treating an inflammatory disorder in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

40. The method of claim 39, wherein said MAP kinase is ERK1/2.

41. The method of claim 39, wherein said target protein is a protein identified in Table 4.

42. The method of claim 39, wherein said inflammatory disorder is selected from the group consisting of anaphylaxis, septic shock, allergic rhinitis, asthma, atopic dermatitis, and food allergies. Examples of autoimmune disorders include, but are not limited to, type 1 insulin-dependent diabetes mellitus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, dermatitis, meningitis, thrombotic thrombocy topenic purpura, Sjögren's syndrome, encephalitis, uveitis, leukocyte adhesion deficiency, rheumatoid and other forms of immune arthritis, rheumatic fever, Reiter's syndrome, psoriatic arthritis, progressive systemic sclerosis, primary biliary cirrhosis, pemphigus, pemphigoid, necrotizing vasculitis, myasthenia gravis, multiple sclerosis, lupus erythematosus, polymyositis, sarcoidosis, granulomatosis, vasculitis, pernicious anemia, CNS inflammatory disorder, antigen-antibody complex mediated diseases, autoimmune hemolytic anemia, Hashimoto's thyroiditis, Graves disease, habitual spontaneous abortions, Reynard's syndrome, glomerulonephritis, dermatomyositis, chronic active hepatitis, celiac disease, autoimmune complications of AIDS, atrophic gastritis, ankylosing spondylitis and Addison's disease.

43. The method of claim 39, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

44. The method of claim 39, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

45. The method of claim 39, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

46. A method for treating a metabolic disorder in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

47. The method of claim 46, wherein said MAP kinase is ERK1/2.

48. The method of claim 46, wherein said target protein is a protein identified in Table 5.

49. The method of claim 46, wherein said metabolic disorder is selected from the group consisting of diabetes, obesity, jaundice, polycystic kidney and hepatic disease, pancreatitis, Graves' disease, and Werner's syndrome.

50. The method of claim 46, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

51. The method of claim 46, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

52. The method of claim 46, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

53. A method for treating a neuropathy or behavioral disorder in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

54. The method of claim 53, wherein said MAP kinase is ERK1/2.

55. The method of claim 53, wherein said target protein is a protein identified in Table 6.

56. The method of claim 53, wherein said neuropathy or behavioral disorder is selected from the group consisting of diabetic neuropathy, muscular dystrophy, Williams Beuren's Syndrome, psychosis, schizophrenia, autism, Down's Syndrome, Parkinson's Disease, Alzheimer's Disease, epilepsy, Cockayne syndrome, bipolar disorders, depression, and opiate addiction.

57. The method of claim 53, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

58. The method of claim 53, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

59. The method of claim 53, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

60. A method for treating a sleep disorder in a mammal, said method comprising administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein.

61. The method of claim 60, wherein said MAP kinase is ERK1/2.

62. The method of claim 60, wherein said target protein is a protein identified in Table 7.

63. The method of claim 60, wherein said sleep disorder is selected from the group consisting of advanced sleep phase disorder, delayed sleep phase disorder, insomnia, and narcolepsy.

64. The method of claim 60, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28).

65. The method of claim 60, wherein said DEF domain comprises the amino acid sequence F/Y-X-F/Y-P (SEQ ID NO: 29).

66. The method of claim 60, wherein said DEF domain comprises the amino acid sequence F-X-F-P (SEQ ID NO: 1).

67. An antibody that specifically binds to phospho-T-325 c-Fos.

68. The antibody of claim 67, wherein said antibody is polyclonal.

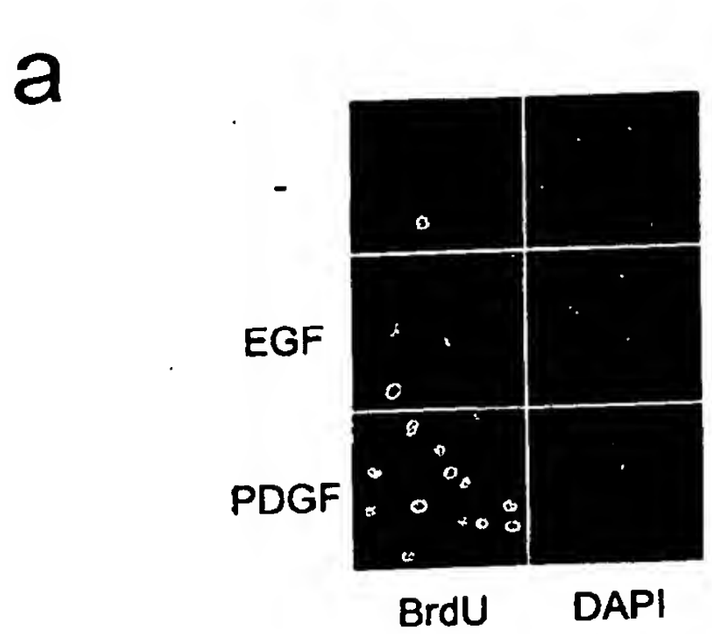
69. The antibody of claim 67, wherein said antibody is monoclonal.

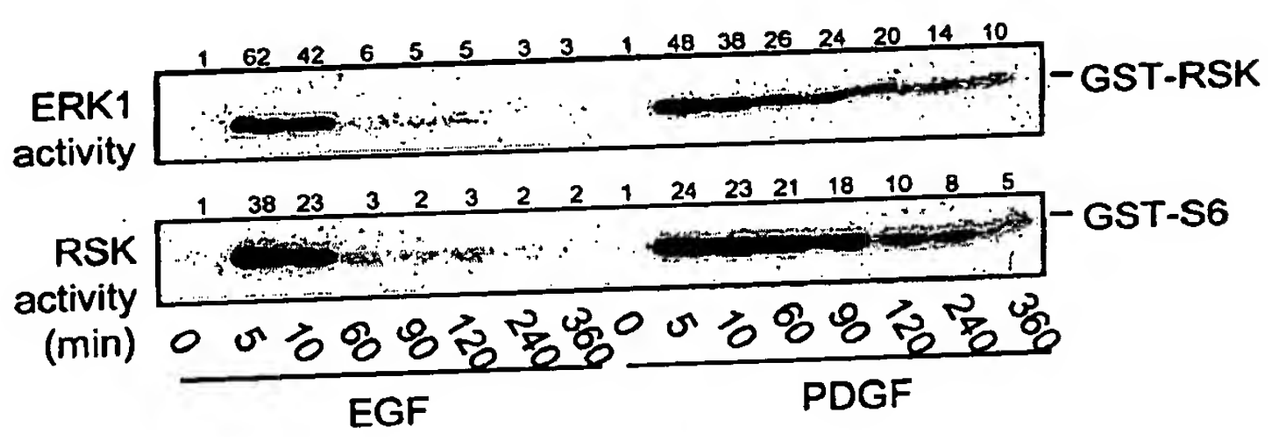
70. A pharmaceutical formulation comprising a therapeutic compound identified by the method of claim 1 or 2 and a pharmaceutically acceptable carrier.

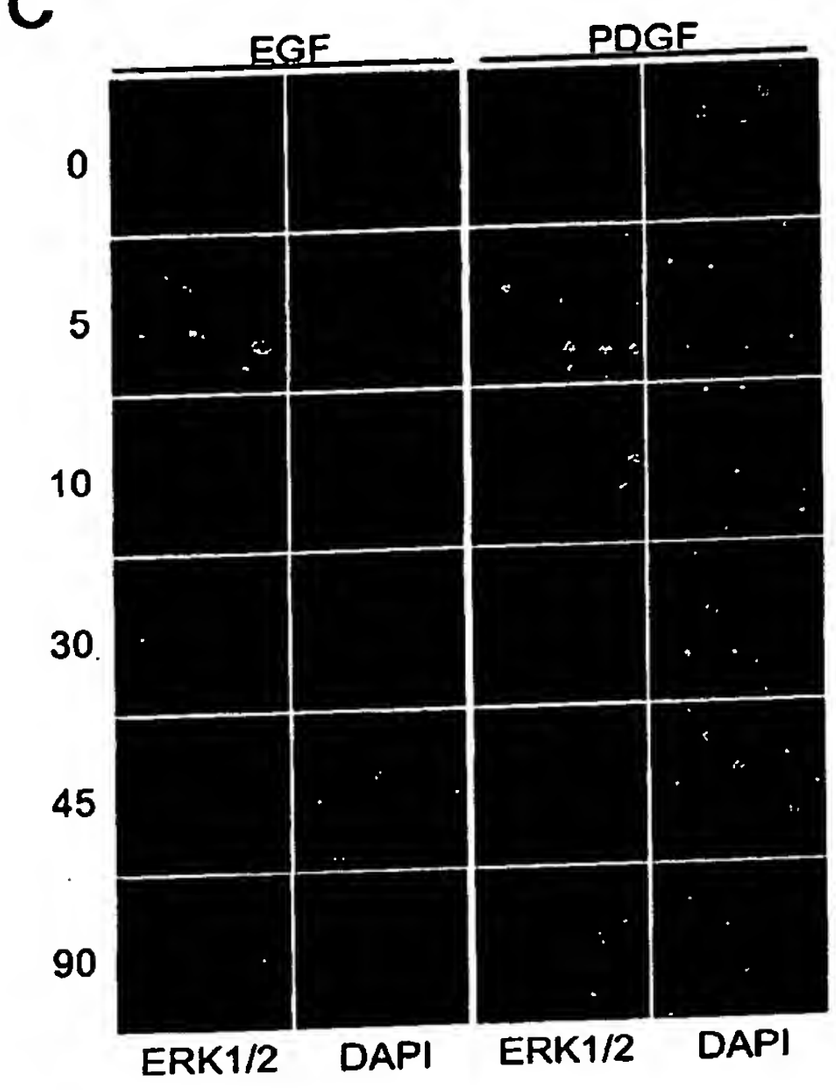
INHIBITORS OF THE MAP KINASE PATHWAY

Abstract of the Disclosure

MAP kinases (e.g., ERK1/2) phosphorylate a variety of target proteins including, for example, several immediate early gene products (e.g., Fos, Myc, and Jun family proteins). Certain phosphorylation reactions require binding of the MAP kinase to the DEF domain of the target protein. Inhibitors that block this interaction may be useful therapeutics for human disease, including as antineoplastic agents. Also disclosed are screening assays useful for identifying compounds that inhibit the MAP kinase-DEF domain interaction.


a
-
EGF
PDGF
BrdU
DAPI


b
1 62 42 6 5 5 3 3 1 48 38 26 24 20 14 10
ERK1 activity
GST-RSK
1 38 23 3 2 3 2 2 1 24 23 21 18 10 8 5
RSK activity
GST-S6
(min)
0 5 10 60 90 120 240 360 0 5 10 60 90 120 240 360
EGF
PDGF


c
EGF
PDGF
0
5
10
30
45
90
ERK1/2
DAPI
ERK1/2
DAPI

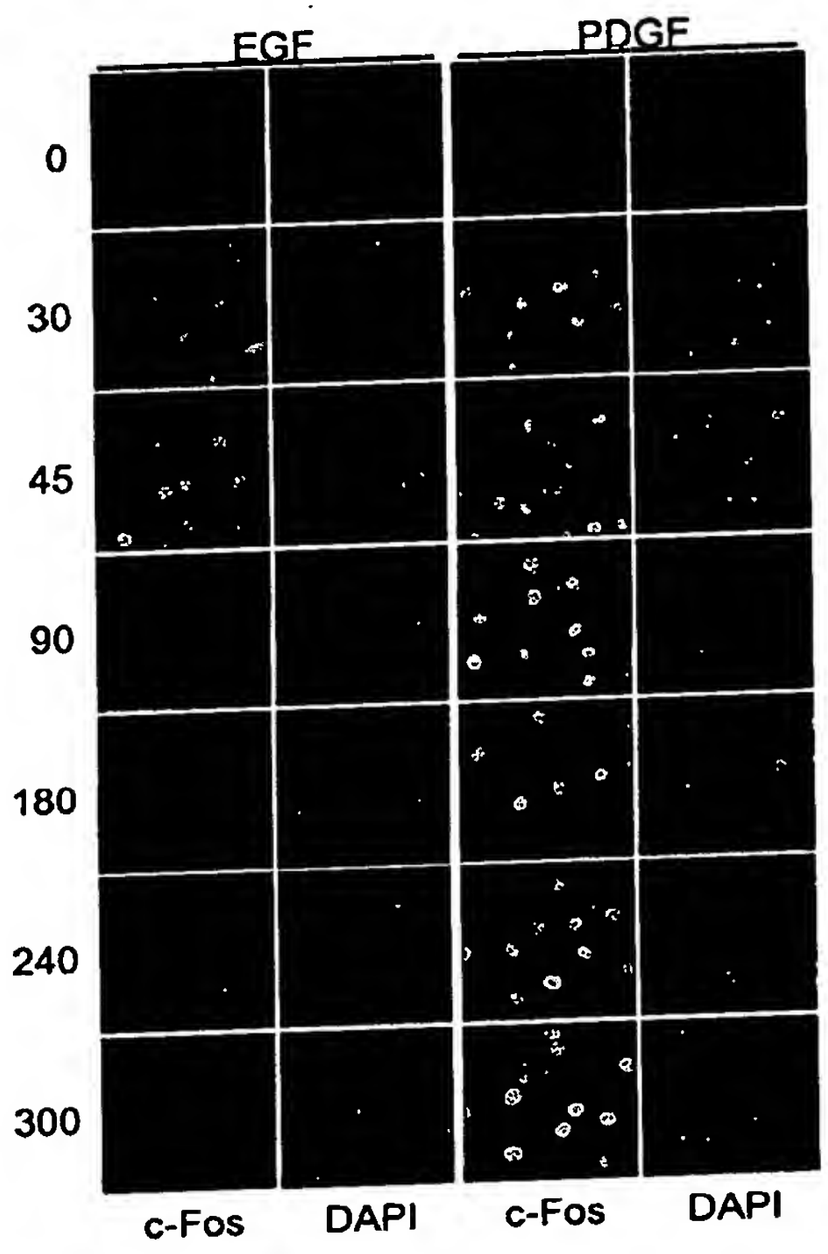

d
EGF
PDGF
0
30
45
90
180
240
300
c-Fos
DAPI
c-Fos
DAPI

Figure 1

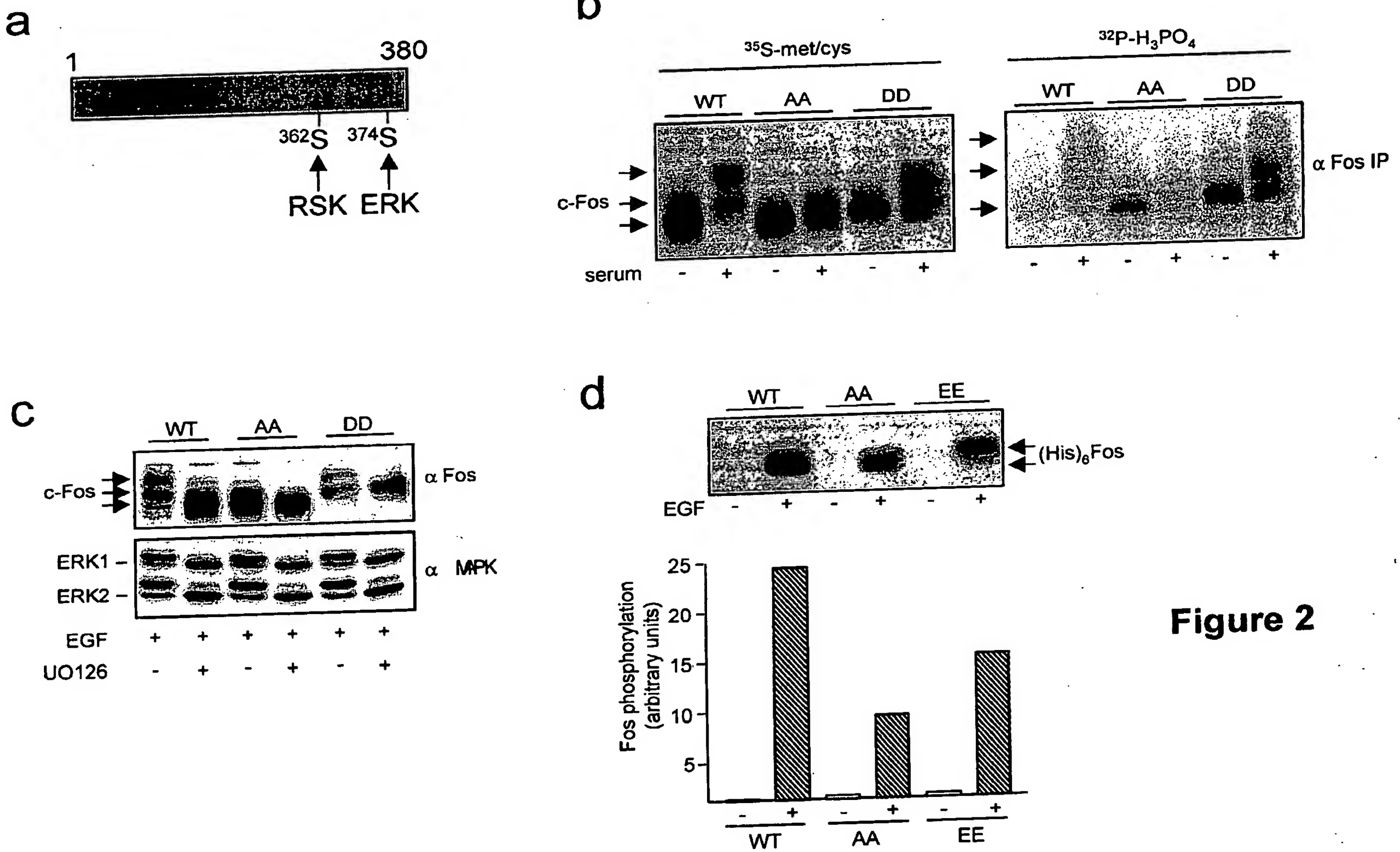
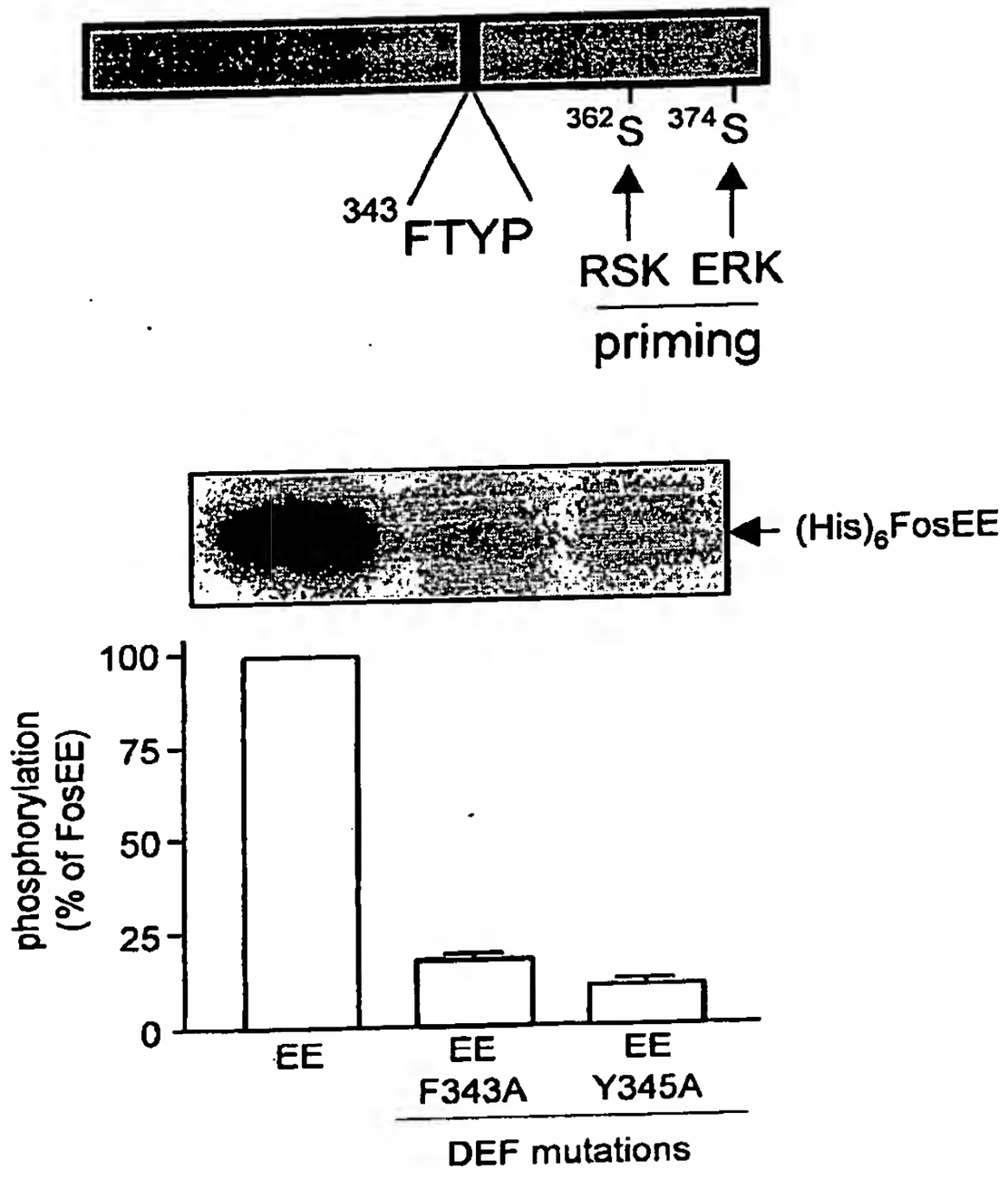
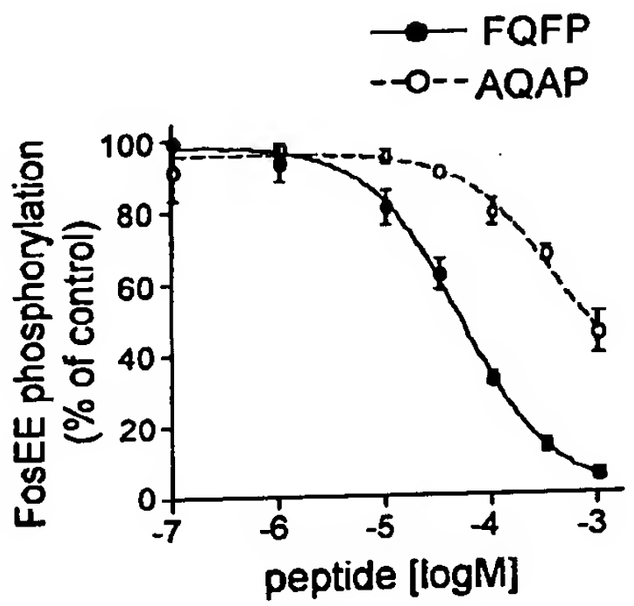

a
1
380
362S
374S
RSK
ERK
b
35S-met/cys
32P-H3PO4
WT
AA
DD
c-Fos
α Fos IP
serum
- + - + - +
c
WT
AA
DD
c-Fos
α Fos
ERK1
ERK2
α MPK
EGF + + + + + +
UO126 - + - + - +
d
WT
AA
EE
(His)6Fos
EGF - + - + - +
Fos phosphorylation (arbitrary units)
25
20
15
10
5
WT
AA
EE

Figure 2

a



b



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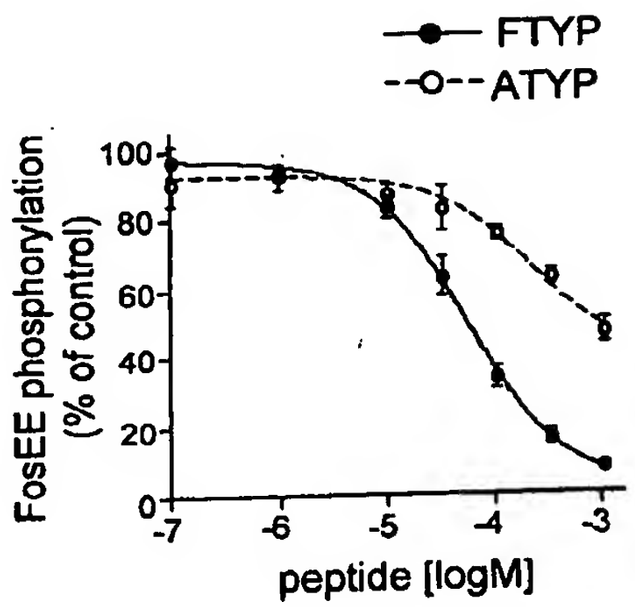
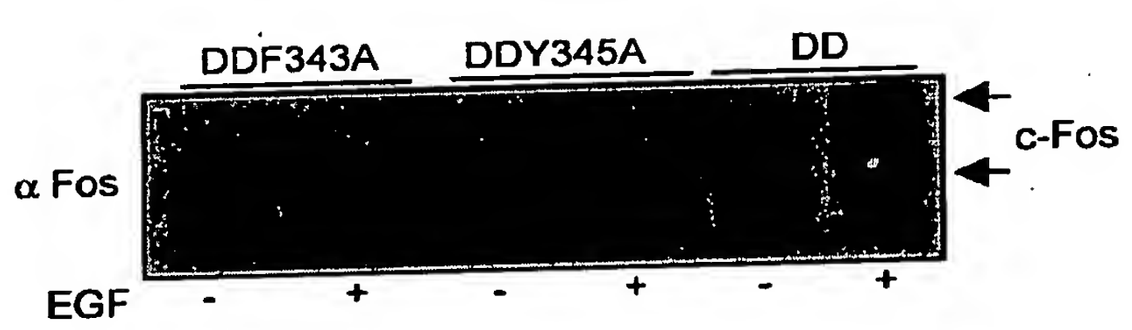


d



e

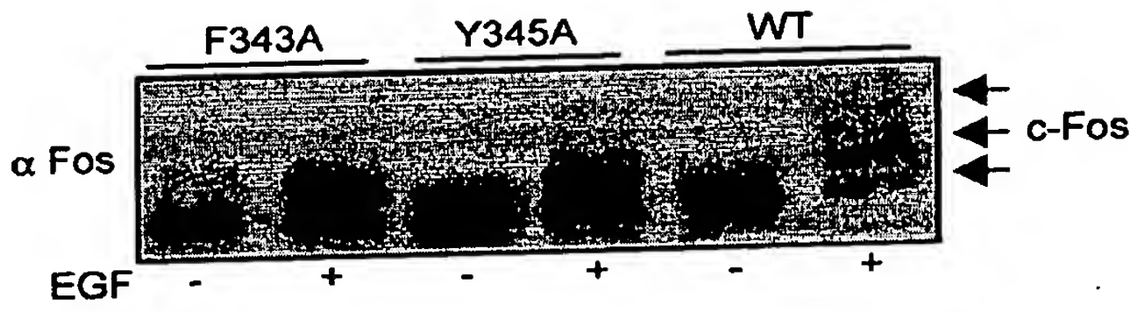


Figure 3

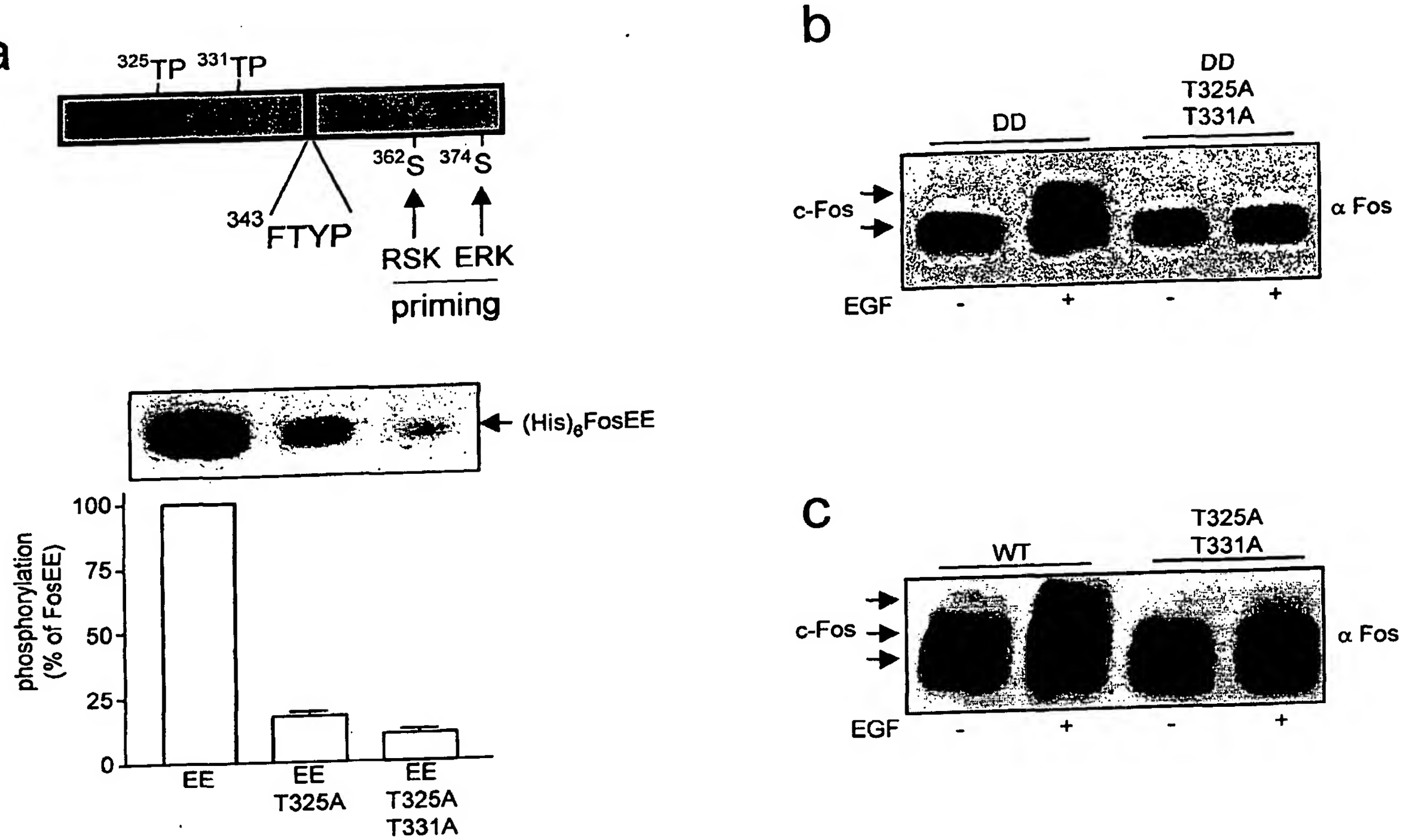

a
325TP 331TP
362S 374S
343FTYP
RSK ERK
priming
(His)6FosEE
phosphorylation (% of FosEE)
100
75
50
25
0
EE
EE T325A
EE T325A T331A
b
DD
DD T325A T331A
c-Fos
α Fos
EGF
- + - +
C
WT
T325A T331A
c-Fos
α Fos
EGF
- + - +

Figure 4

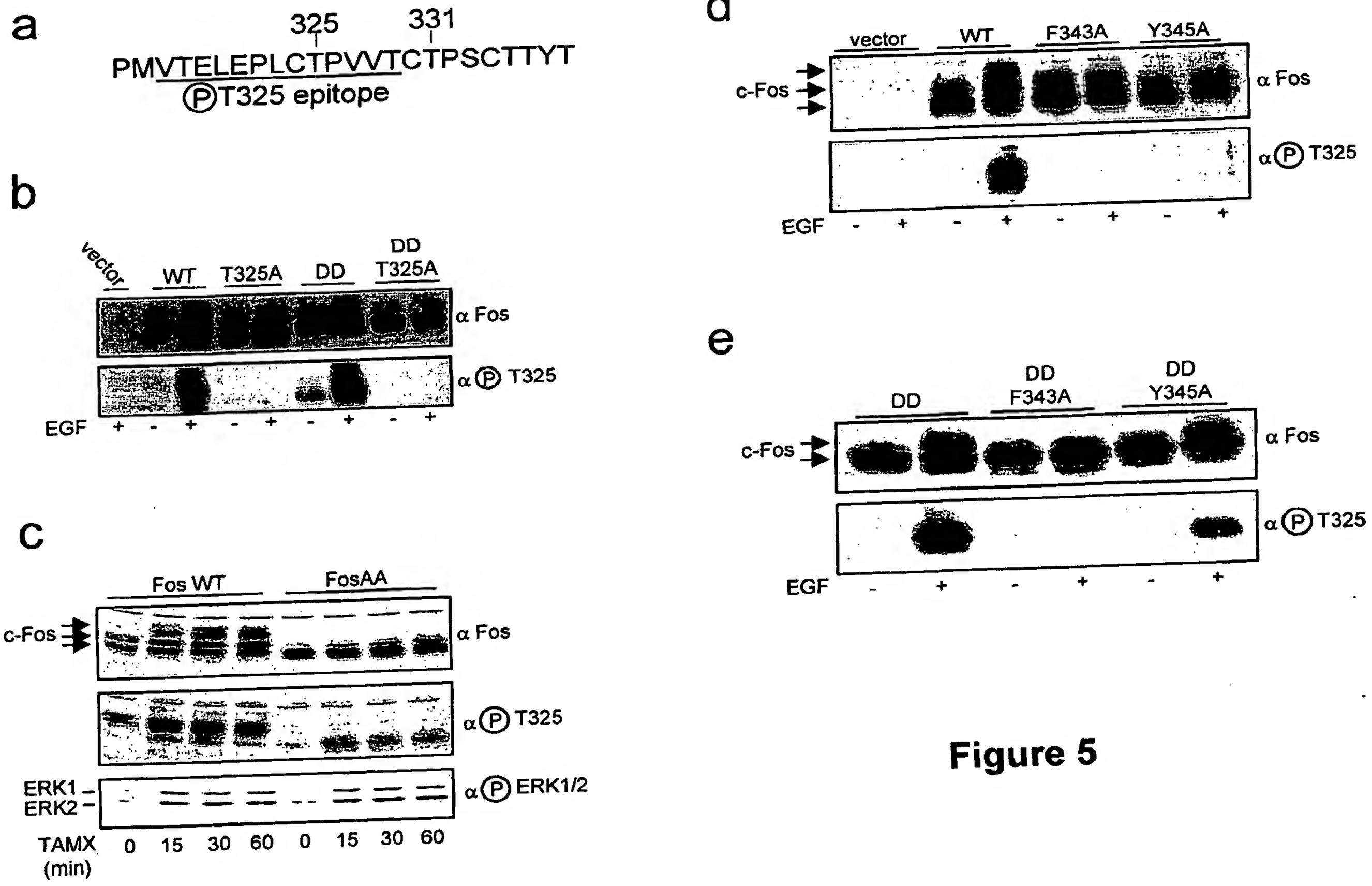

a
325
331
PMVTELEPLCTPVVTCTPSCTTYT
ⓅT325 epitope
b
vector
WT
T325A
DD
DD T325A
α Fos
α Ⓟ T325
EGF + - + - + - + - +
C
Fos WT
FosAA
c-Fos
α Fos
α Ⓟ T325
ERK1
ERK2
α Ⓟ ERK1/2
TAMX (min) 0 15 30 60 0 15 30 60
d
vector
WT
F343A
Y345A
c-Fos
α Fos
α Ⓟ T325
EGF - + - + - + - +
e
DD
DD F343A
DD Y345A
c-Fos
α Fos
α Ⓟ T325
EGF - + - + - +

Figure 5

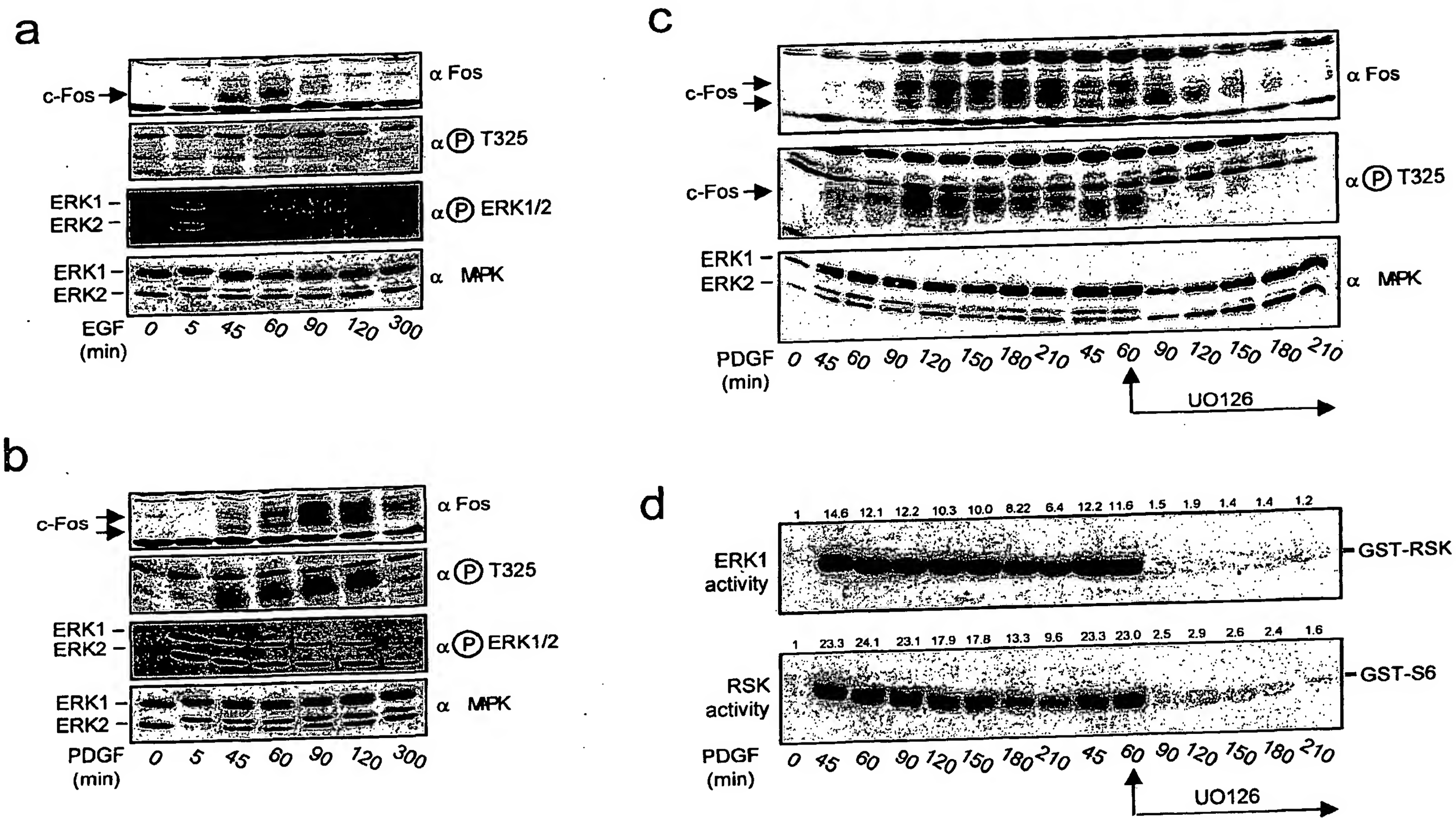
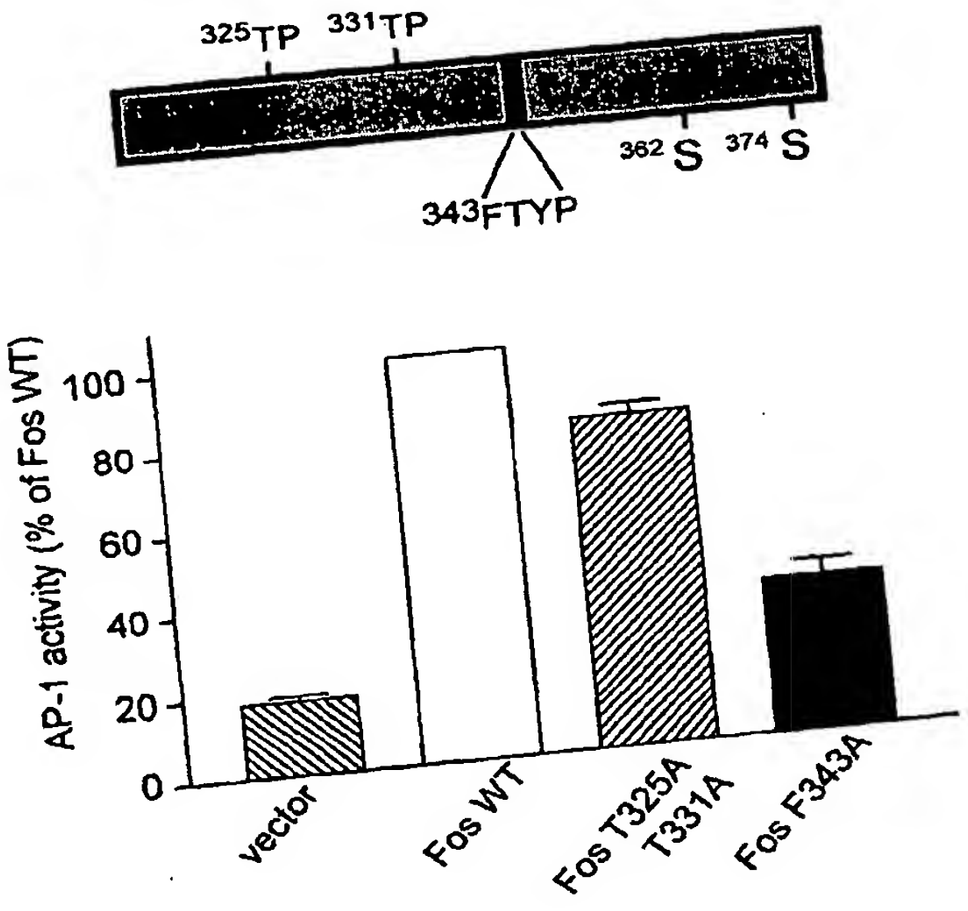
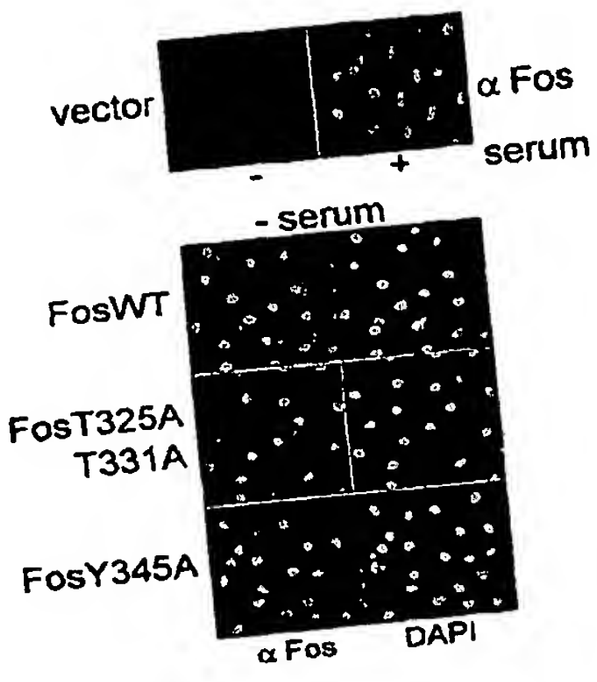

a
c-Fos
α Fos
α Ⓟ T325
ERK1
ERK2
α Ⓟ ERK1/2
ERK1
ERK2
α MPK
EGF (min) 0 5 45 60 90 120 300
b
c-Fos
α Fos
α Ⓟ T325
ERK1
ERK2
α Ⓟ ERK1/2
ERK1
ERK2
α MPK
PDGF (min) 0 5 45 60 90 120 300
c
c-Fos
α Fos
c-Fos
α Ⓟ T325
ERK1
ERK2
α MPK
PDGF (min) 0 45 60 90 120 150 180 210 45 60 90 120 150 180 210
UO126
d
1 14.6 12.1 12.2 10.3 10.0 8.22 6.4 12.2 11.6 1.5 1.9 1.4 1.4 1.2
ERK1 activity
GST-RSK
1 23.3 24.1 23.1 17.9 17.8 13.3 9.6 23.3 23.0 2.5 2.9 2.6 2.4 1.6
RSK activity
GST-S6
PDGF (min) 0 45 60 90 120 150 180 210 45 60 90 120 150 180 210
UO126

Figure 6

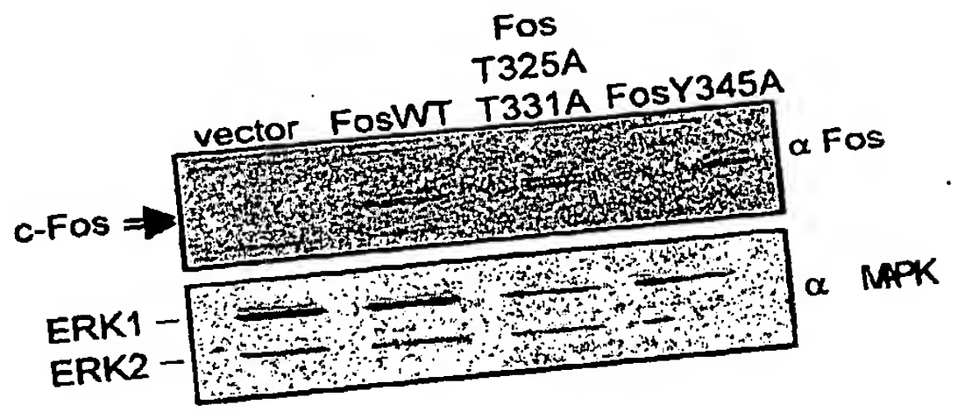
a



b



c



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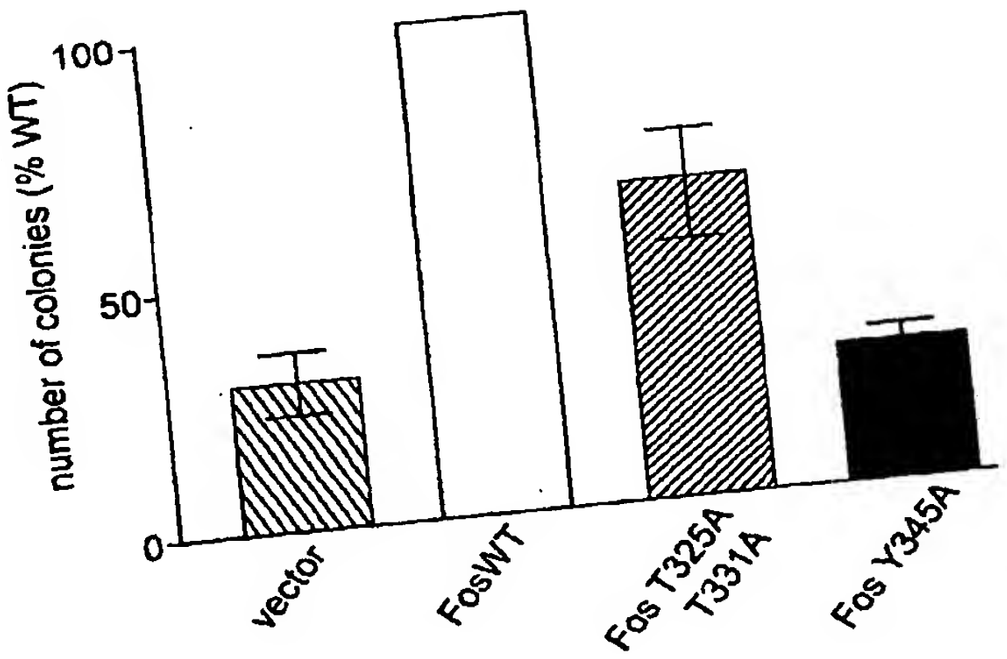
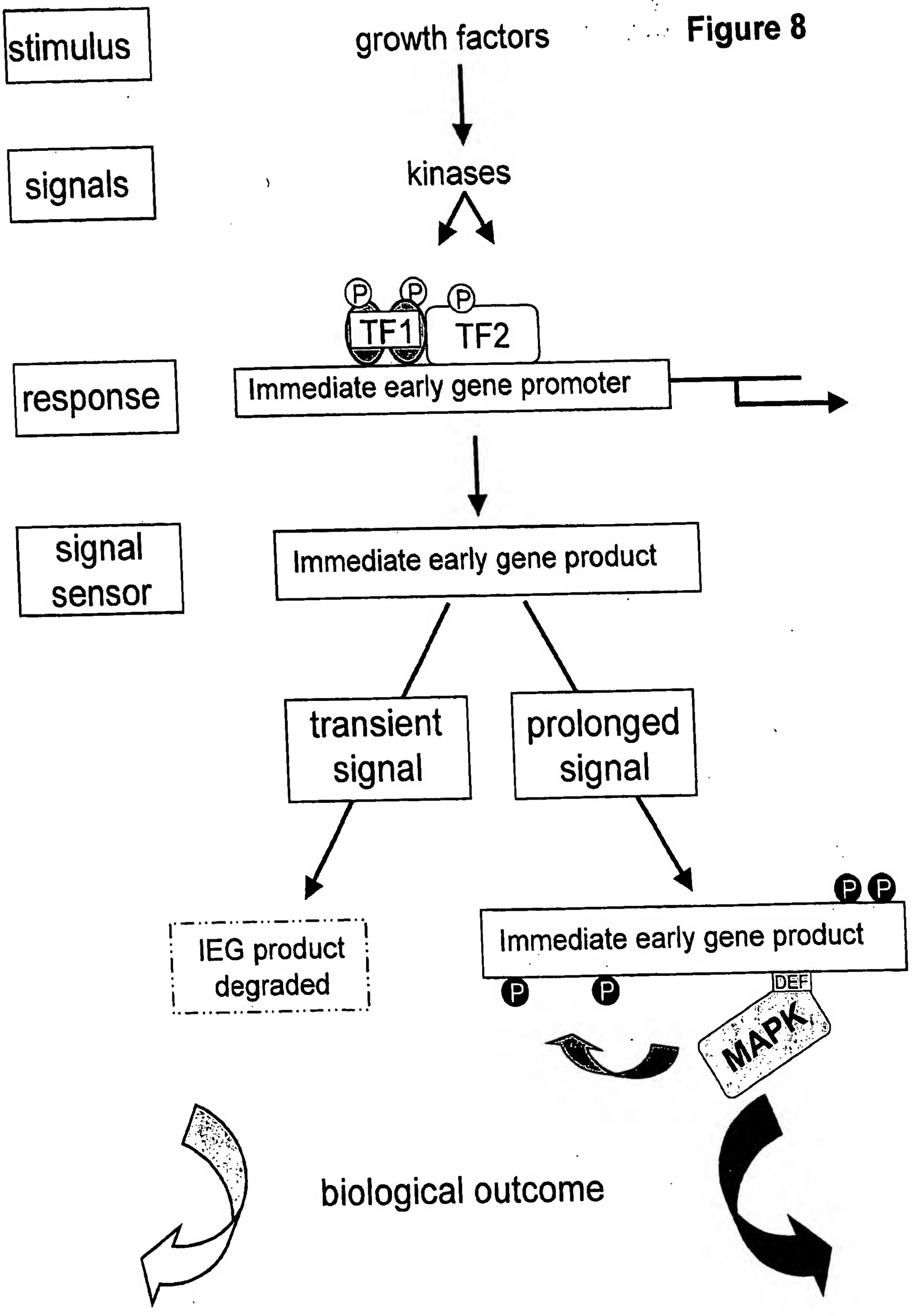


Figure 7

Figure 8


stimulus
growth factors
signals
kinases
P
P
P
TF1
TF2
response
Immediate early gene promoter
signal sensor
Immediate early gene product
transient signal
prolonged signal
IEG product degraded
P
P
Immediate early gene product
P
P
DEF
MAPK
biological outcome

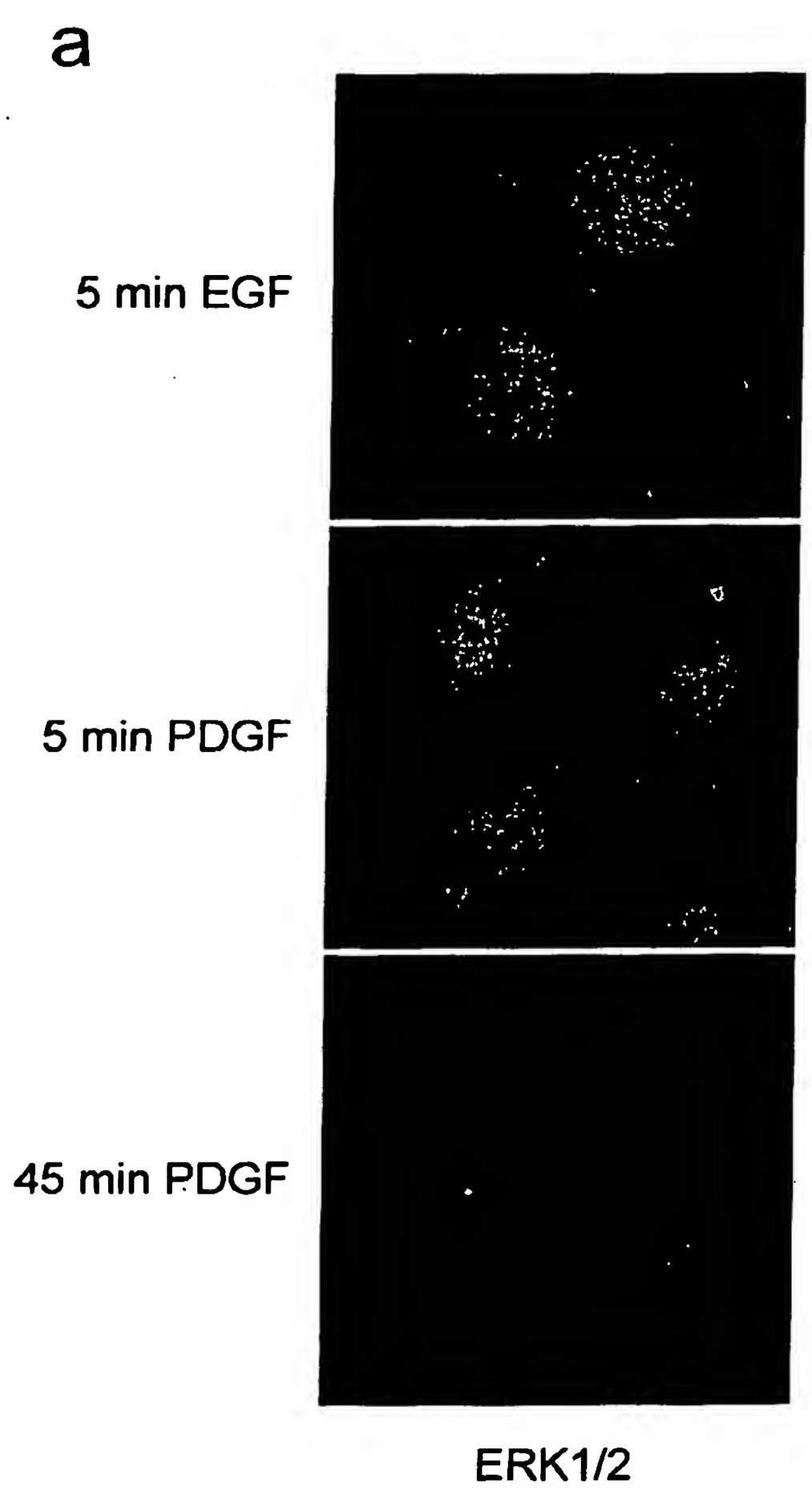
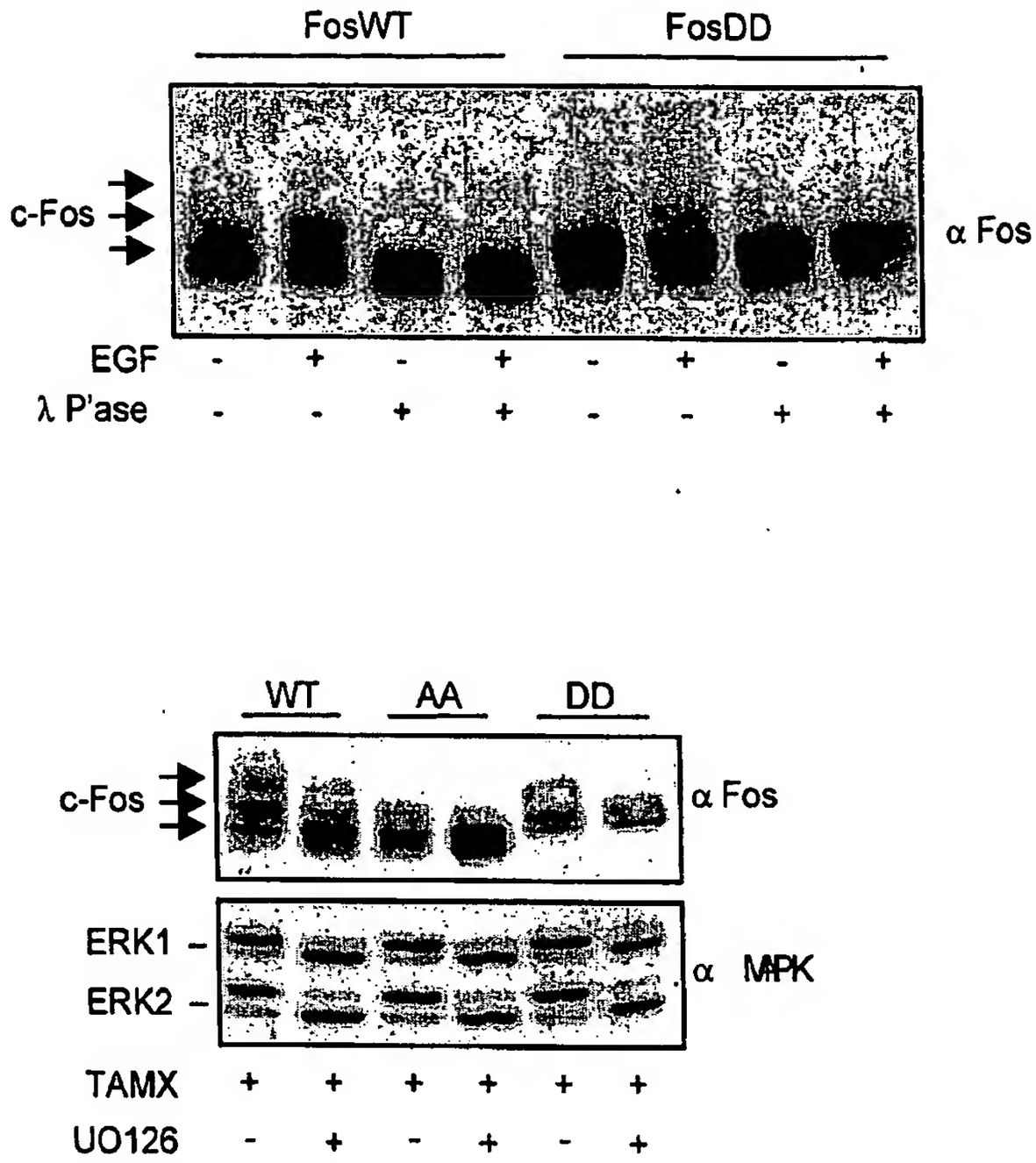

a
5 min EGF
5 min PDGF
45 min PDGF
ERK1/2
b
PDGF
-
+
CHX (added 75 min after PDGF)
90
180
300
-
+
CHX (added 20 min before PDGF)
90
c-Fos

Figure 9

Figure 10

a



b

c

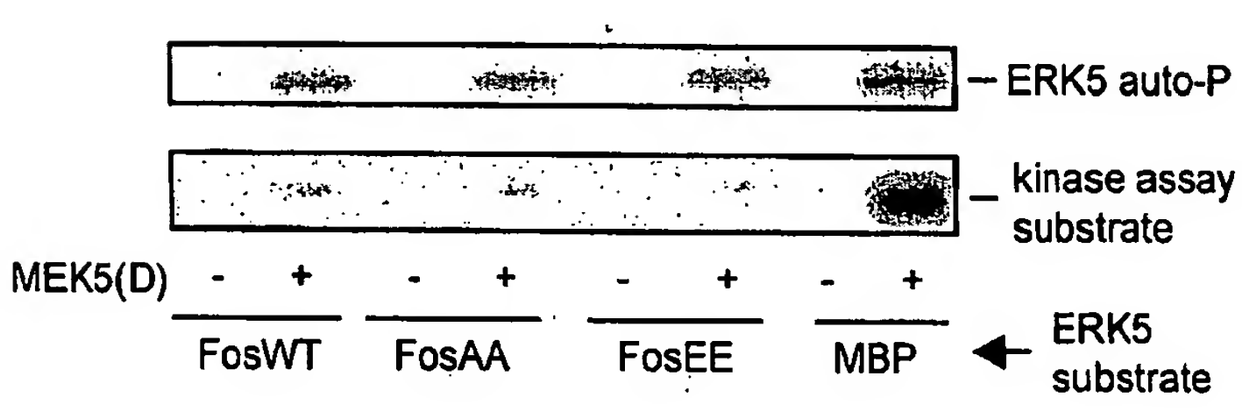


Figure 11

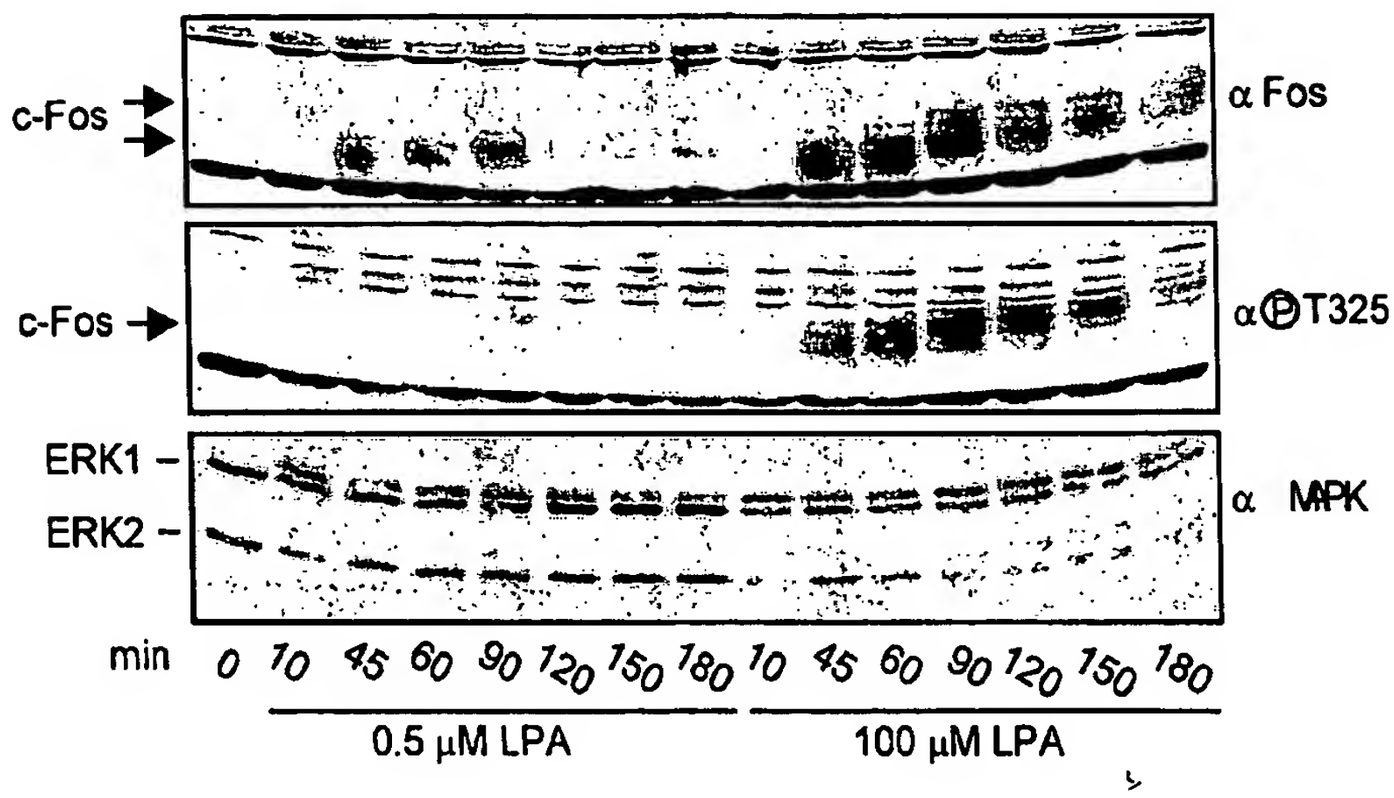
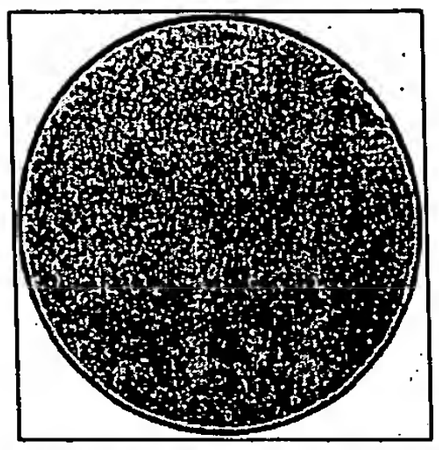
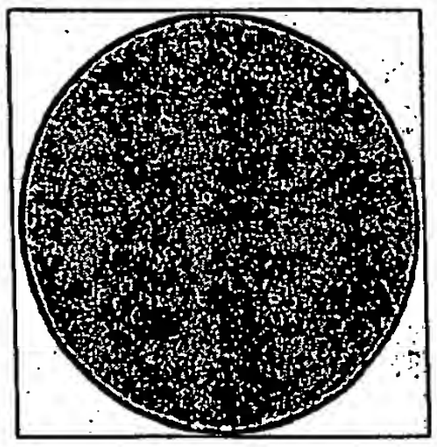
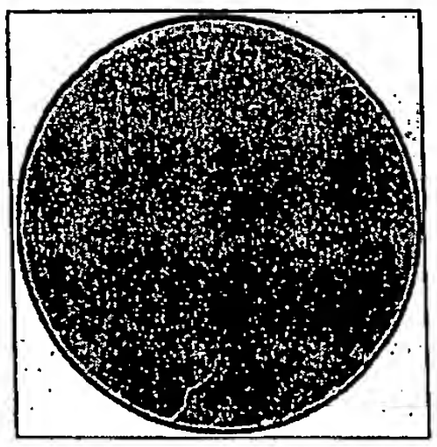
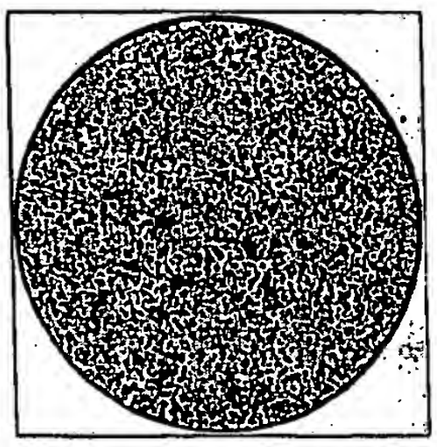

c-Fos
α Fos
c-Fos
αⓅT325
ERK1
ERK2
α MAPK
min 0 10 45 60 90 120 150 180 10 45 60 90 120 150 180
0.5 μM LPA
100 μM LPA

Figure 12









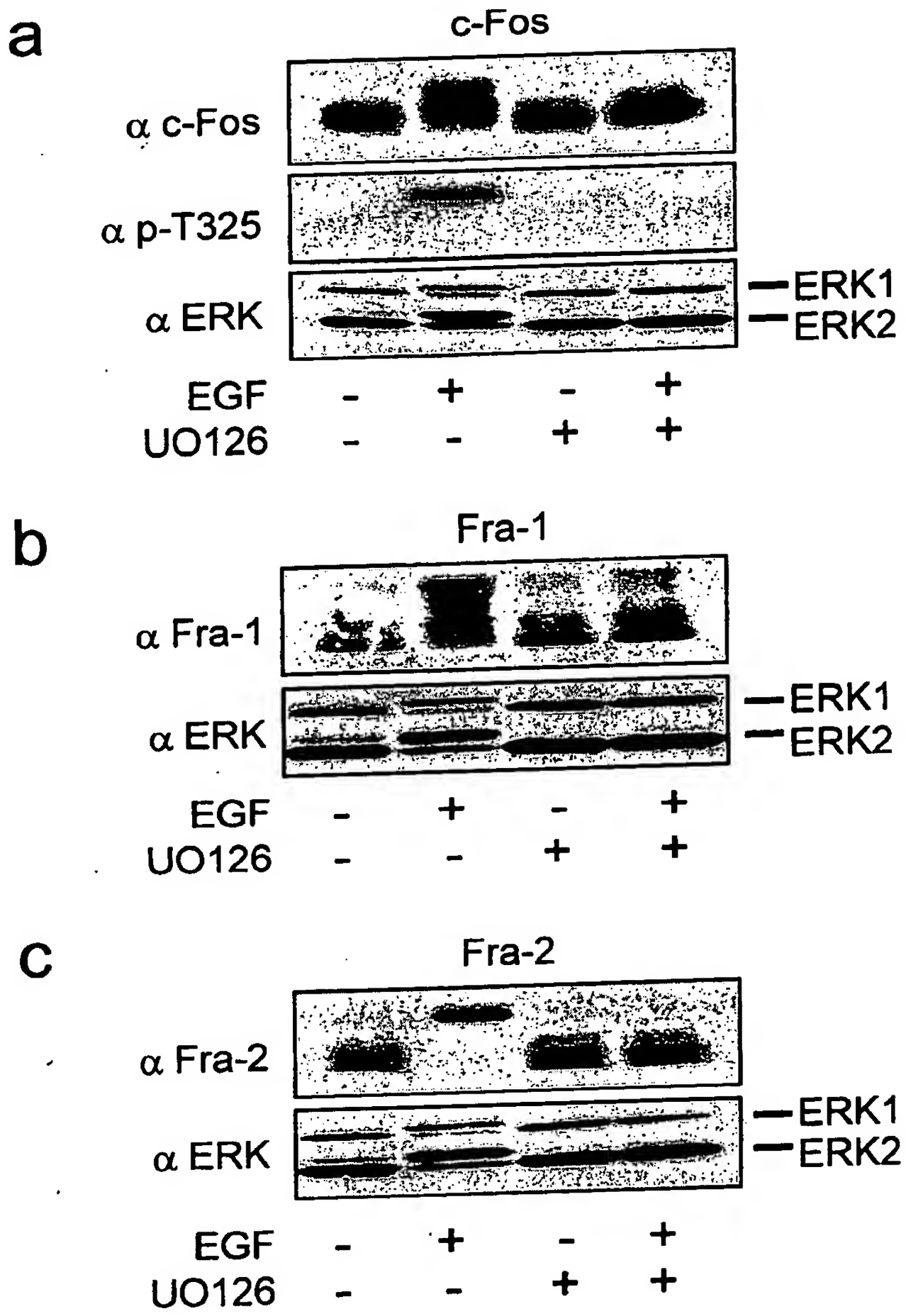

a
c-Fos
α c-Fos
α p-T325
α ERK
ERK1
ERK2
EGF - + - +
UO126 - - + +
b
Fra-1
α Fra-1
α ERK
ERK1
ERK2
EGF - + - +
UO126 - - + +
C
Fra-2
α Fra-2
α ERK
ERK1
ERK2
EGF - + - +
UO126 - - + +

Figure 13

a

```
c-Fos GPMVTELEPLCTPVVTCTPSCTTYTSSFVFTYP-------EADSFPSCAAAHRKGSSS-NEPSSDSLSSPTLLAL
Fra-2 ----GEEPLHTPIVVTSTPAVTPGTSNLVFTYPSVLEQESPASPSESCSKAHRRSSSS-GDQSSDSLNSPTLLAL
Fra-1 -GPVLEPEALHTPTLMTTPSLTPFTPSLVFTYP---------STPEPCSSTHRKSSSSSGDPSSDPLGSPTLLAL
```

b

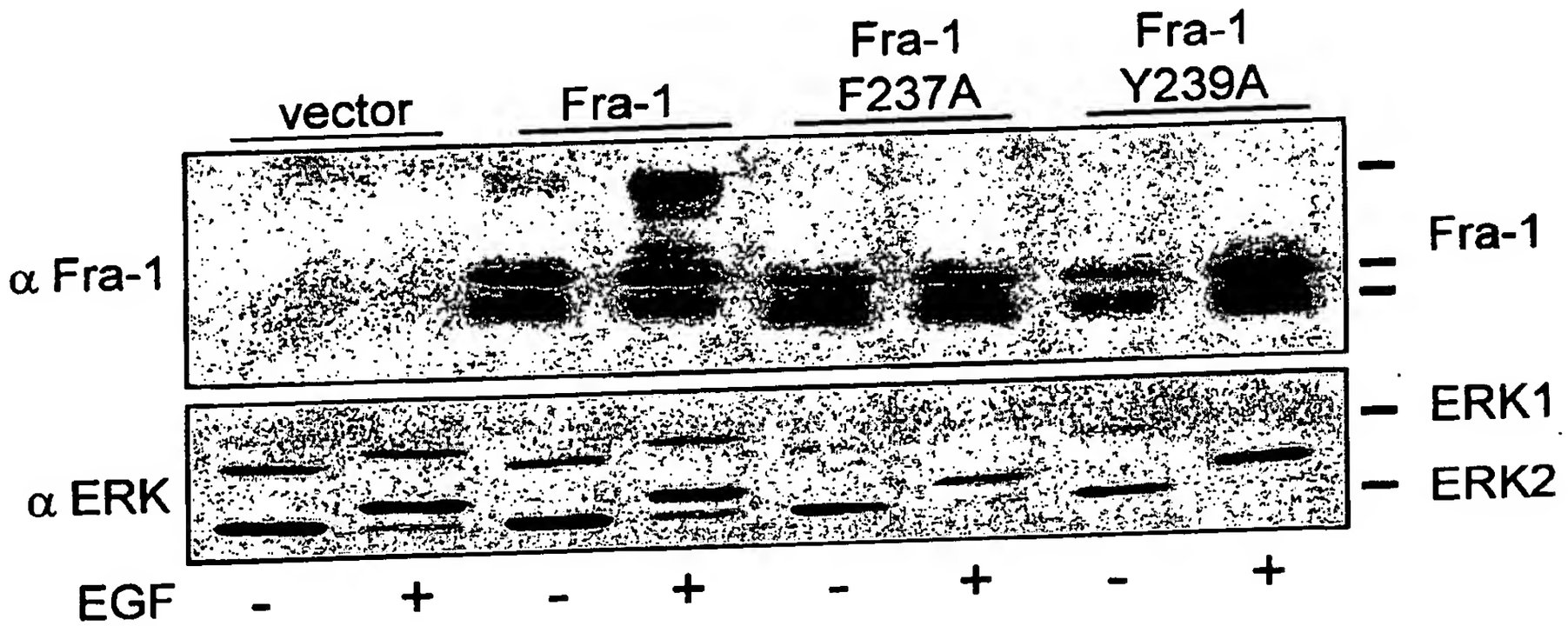
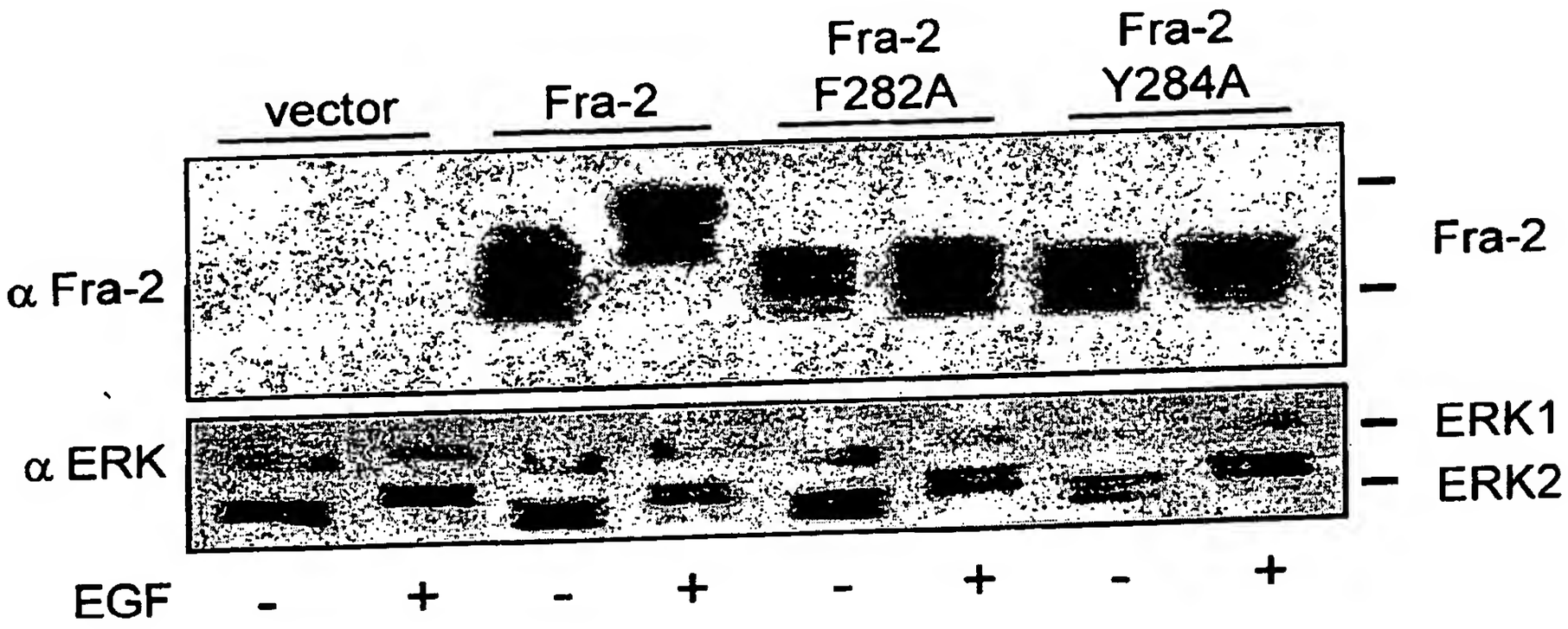


Figure 14

C



a

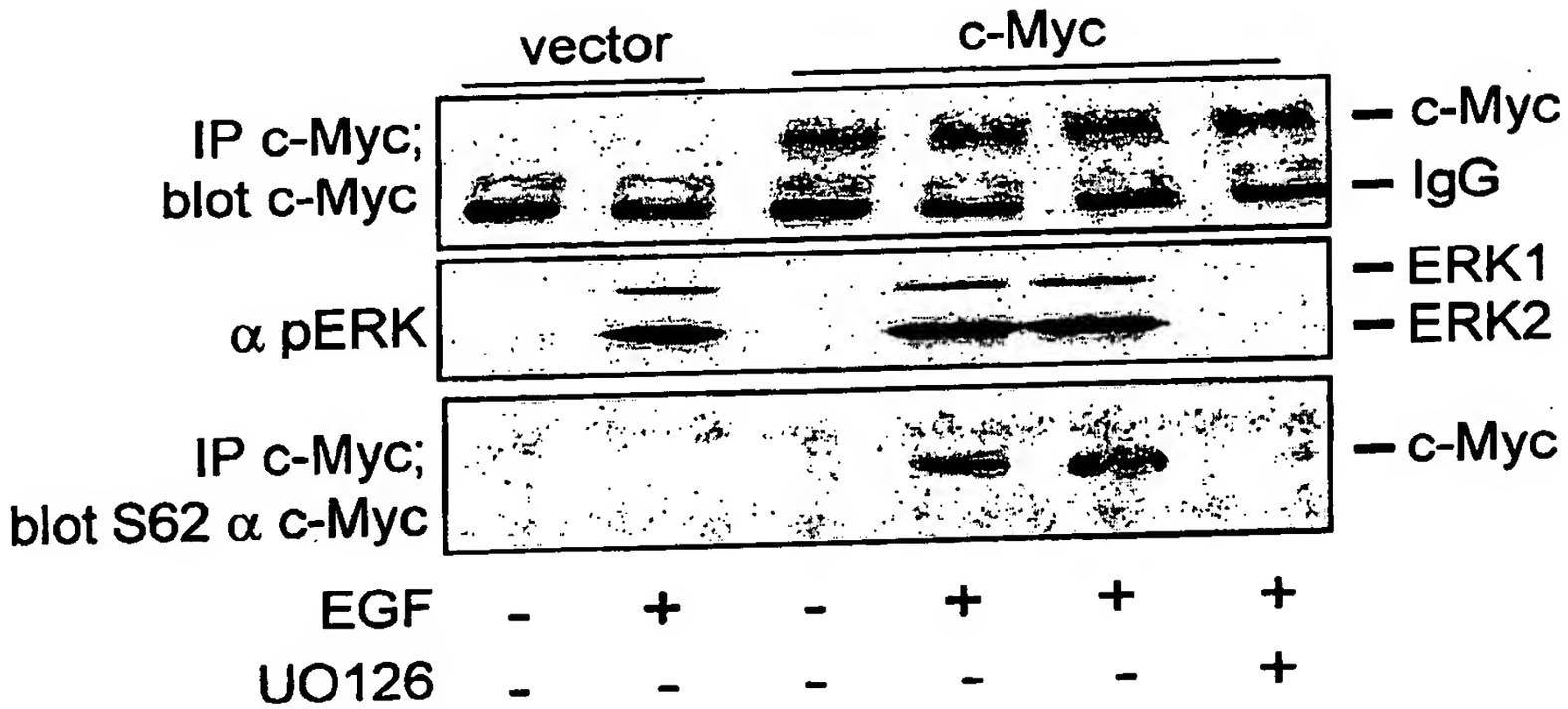
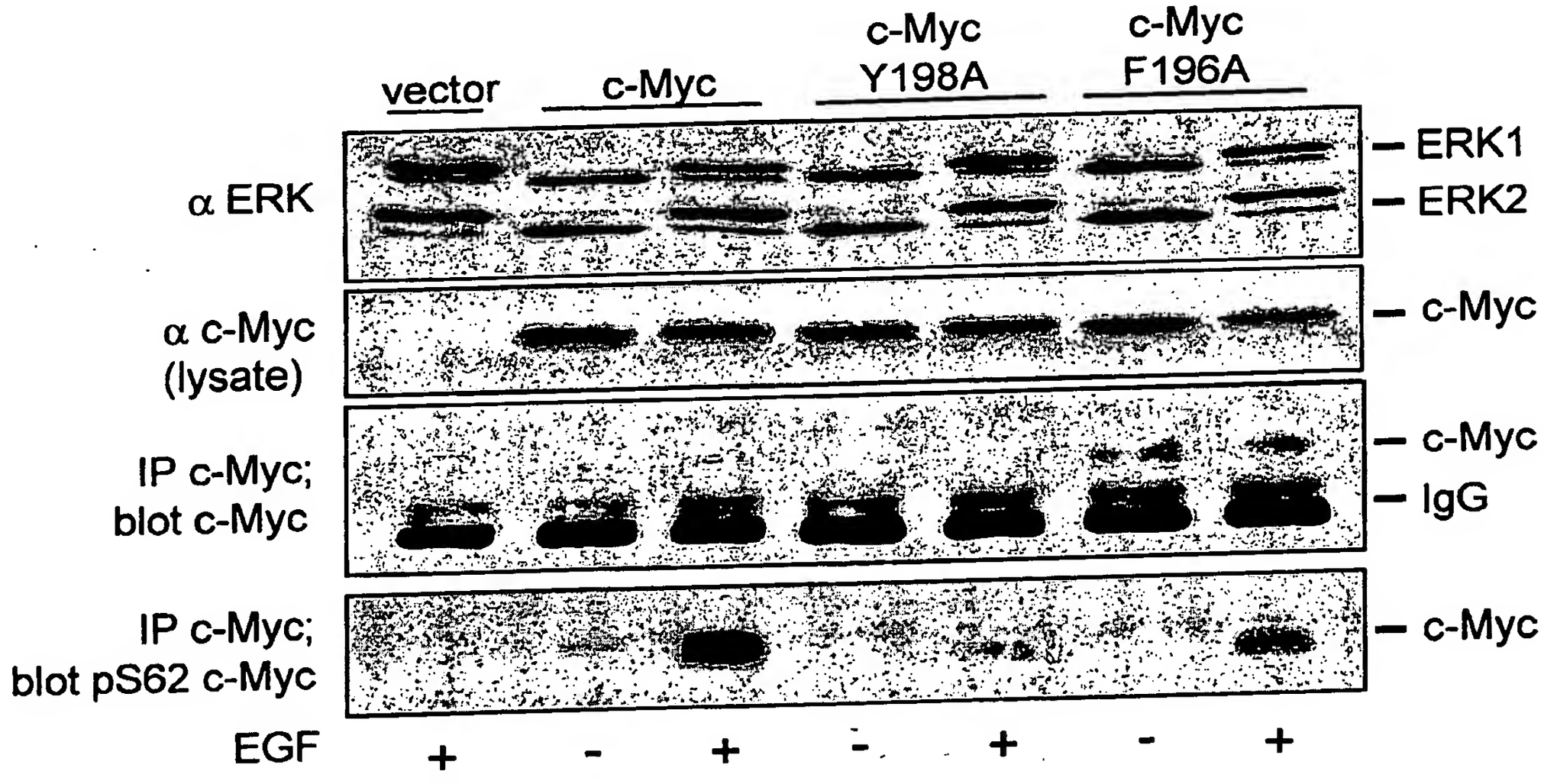

vector
c-Myc
IP c-Myc;
blot c-Myc
c-Myc
IgG
α pERK
ERK1
ERK2
IP c-Myc;
blot S62 α c-Myc
c-Myc
EGF - + - + + +
UO126 - - - - - +

FIGURE 15

b


vector
c-Myc
c-Myc Y198A
c-Myc F196A
α ERK
ERK1
ERK2
α c-Myc (lysate)
c-Myc
IP c-Myc; blot c-Myc
c-Myc
IgG
IP c-Myc; blot pS62 c-Myc
c-Myc
EGF + - + - + - +

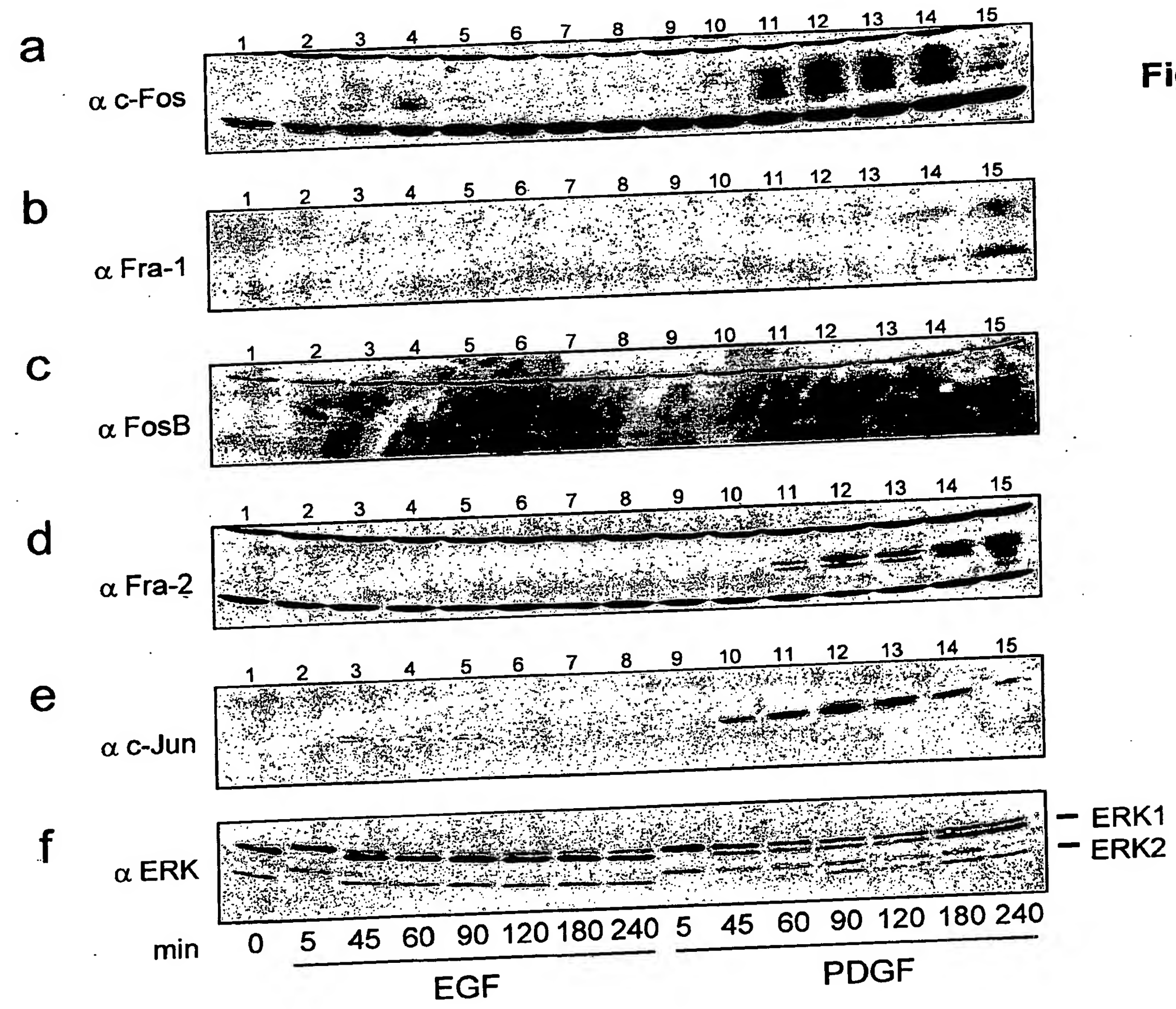
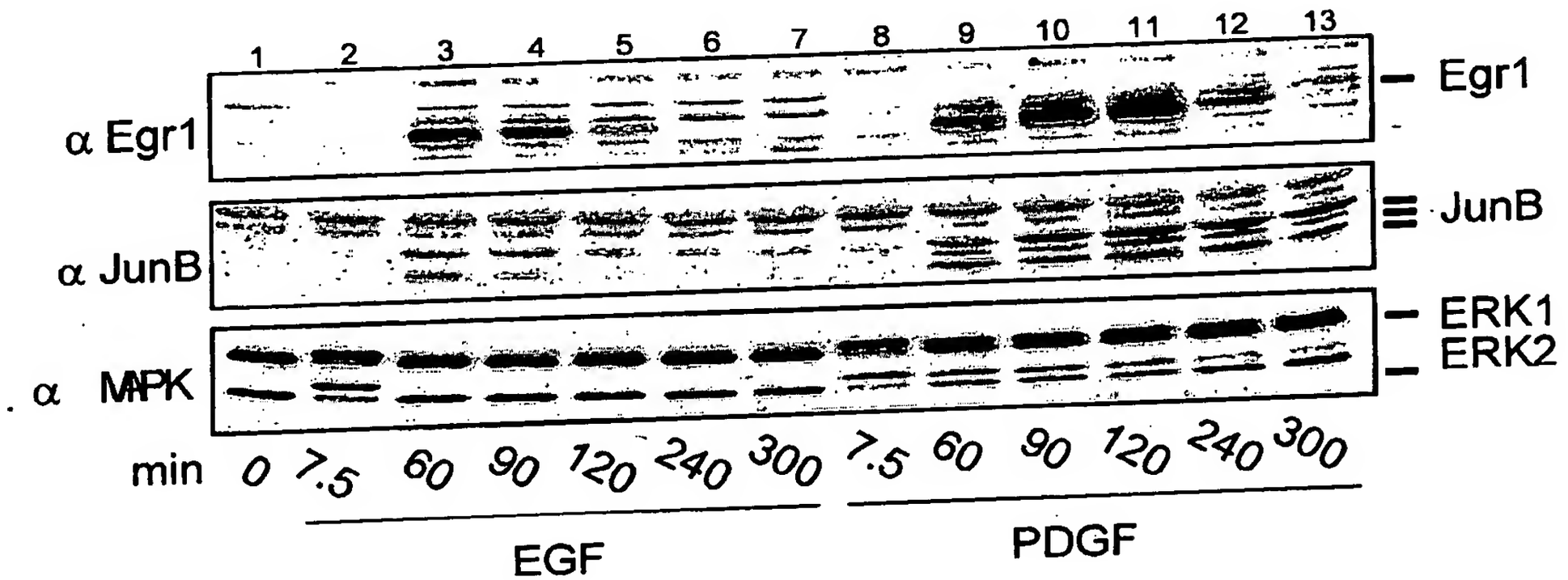

a
α c-Fos
b
α Fra-1
c
α FosB
d
α Fra-2
e
α c-Jun
f
α ERK
ERK1
ERK2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
min 0 5 45 60 90 120 180 240 5 45 60 90 120 180 240
EGF
PDGF

Figure 16

Figure 17

a


1 2 3 4 5 6 7 8 9 10 11 12 13
α Egr1
α JunB
α MAPK
Egr1
JunB
ERK1
ERK2
min 0 7.5 60 90 120 240 300 7.5 60 90 120 240 300
EGF
PDGF

b

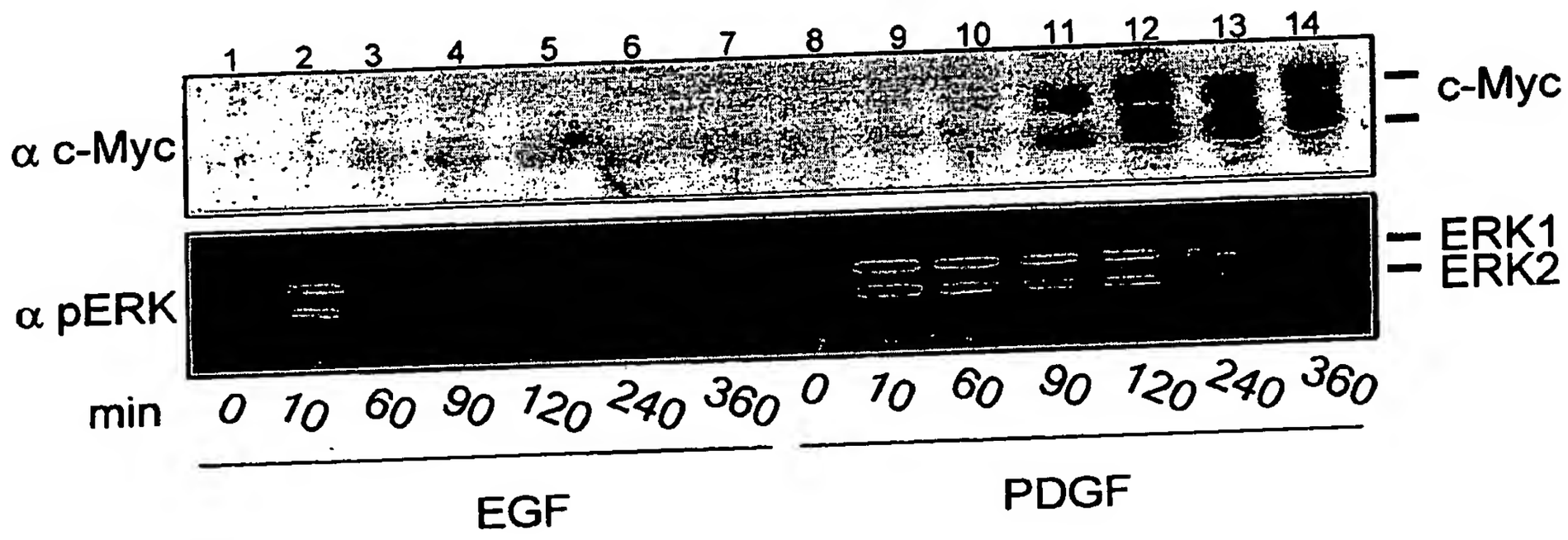
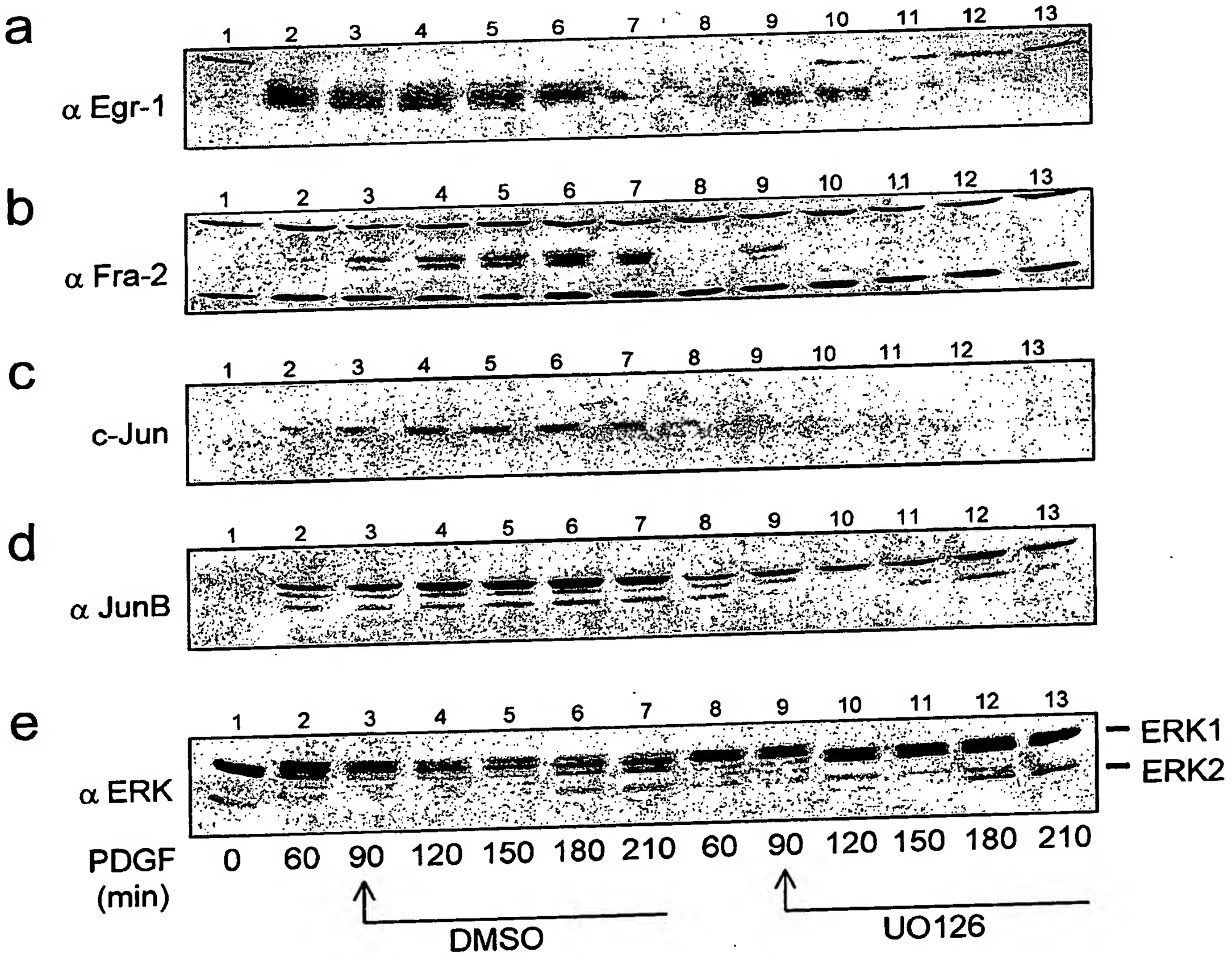
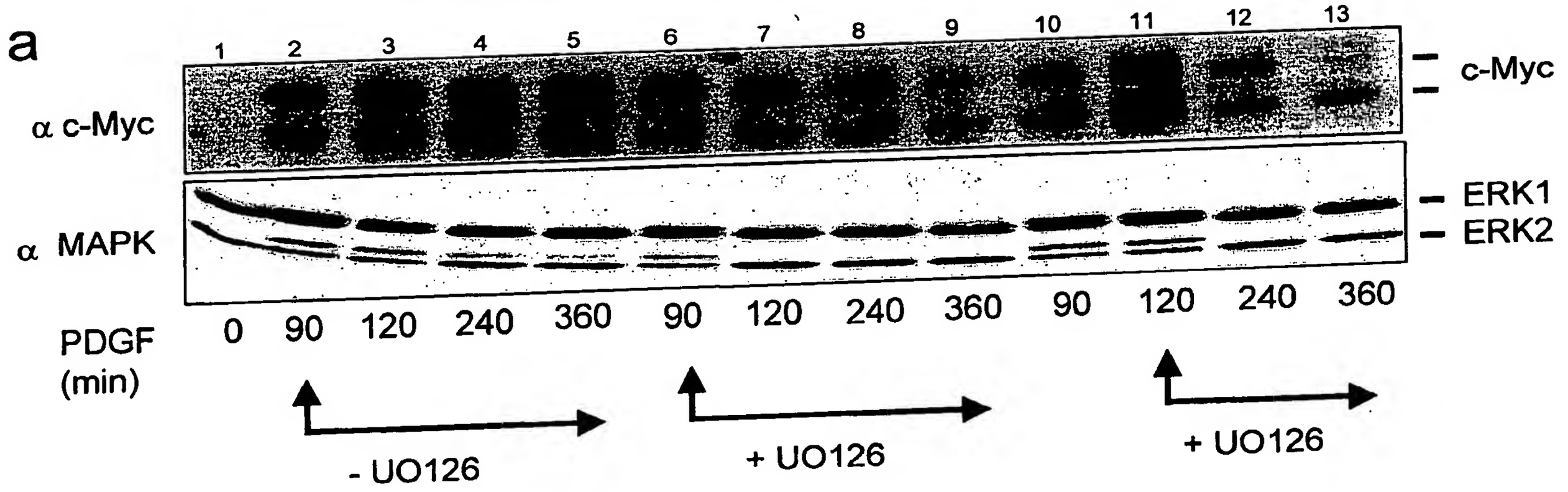

1 2 3 4 5 6 7 8 9 10 11 12 13 14
α c-Myc
α pERK
c-Myc
ERK1
ERK2
min 0 10 60 90 120 240 360 0 10 60 90 120 240 360
EGF
PDGF

Figure 18


a
1 2 3 4 5 6 7 8 9 10 11 12 13
α Egr-1
b
1 2 3 4 5 6 7 8 9 10 11 12 13
α Fra-2
c
1 2 3 4 5 6 7 8 9 10 11 12 13
c-Jun
d
1 2 3 4 5 6 7 8 9 10 11 12 13
α JunB
e
1 2 3 4 5 6 7 8 9 10 11 12 13
α ERK
ERK1
ERK2
PDGF 0 60 90 120 150 180 210 60 90 120 150 180 210
(min)
DMSO
UO126


a
1 2 3 4 5 6 7 8 9 10 11 12 13
α c-Myc
c-Myc
α MAPK
ERK1
ERK2
PDGF (min)
0 90 120 240 360 90 120 240 360 90 120 240 360
- UO126
+ UO126
+ UO126

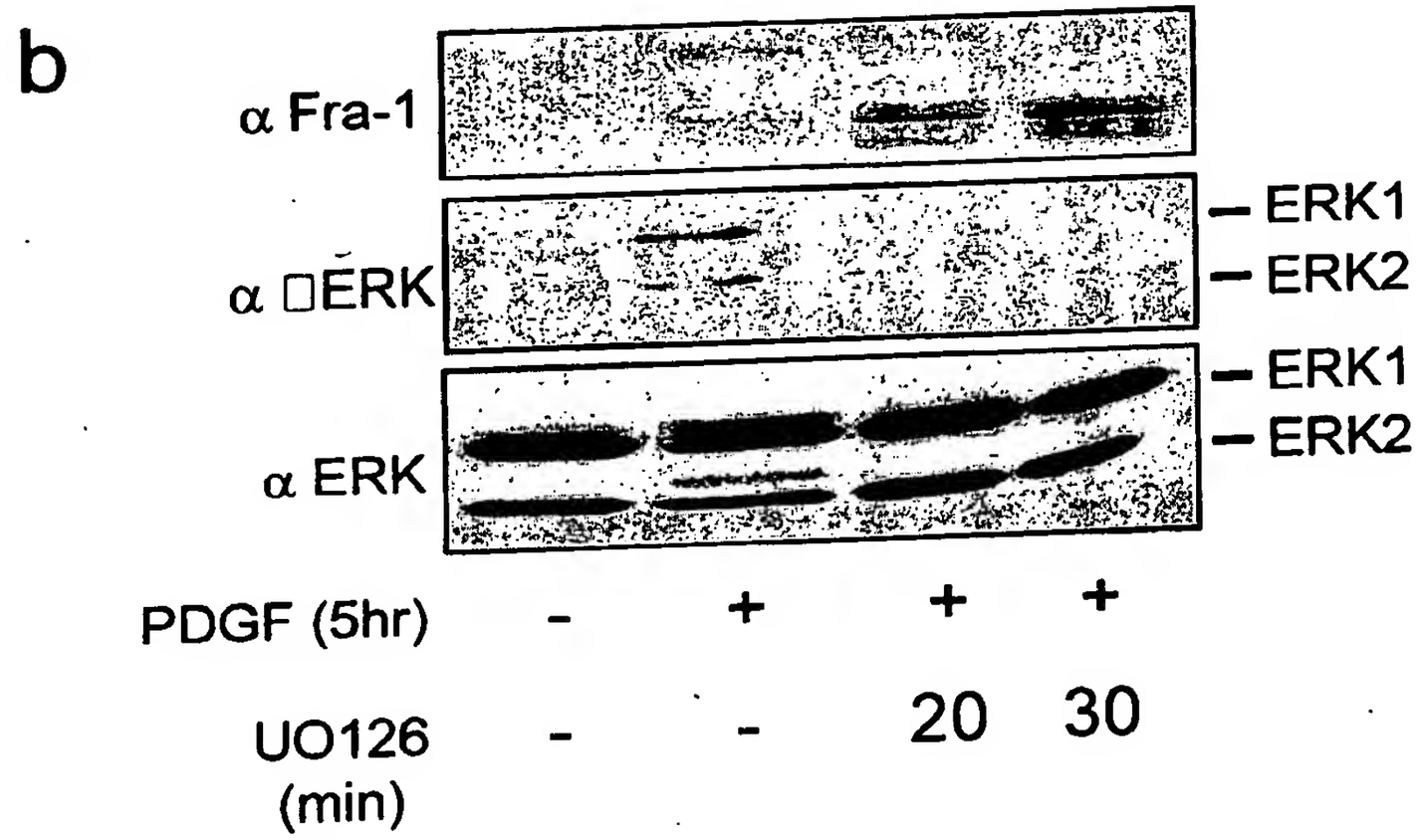

b
α Fra-1
α ERK
ERK1
ERK2
α ERK
ERK1
ERK2
PDGF (5hr) - + + +
UO126 (min) - - 20 30

Figure 19

Figure 20

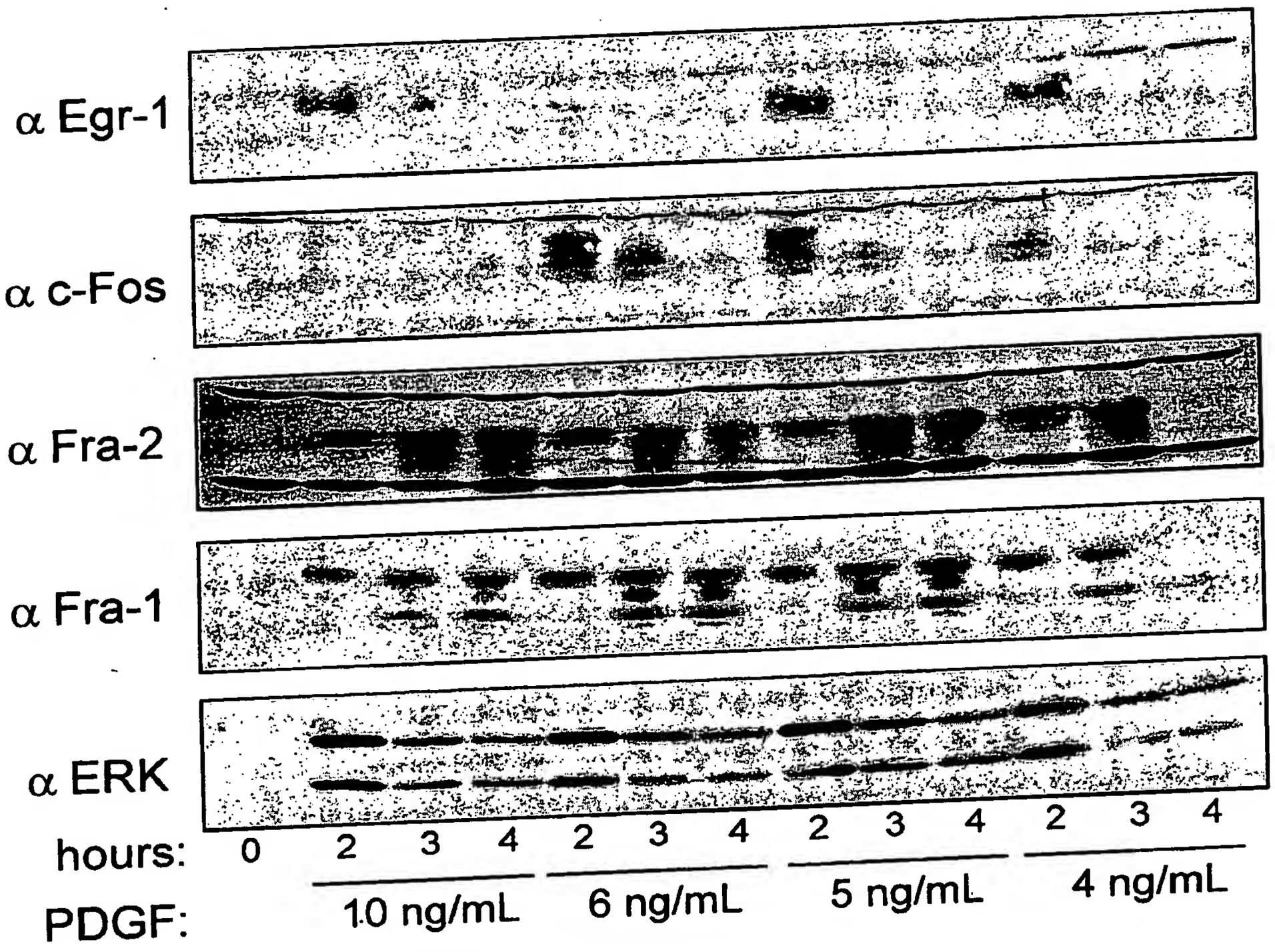
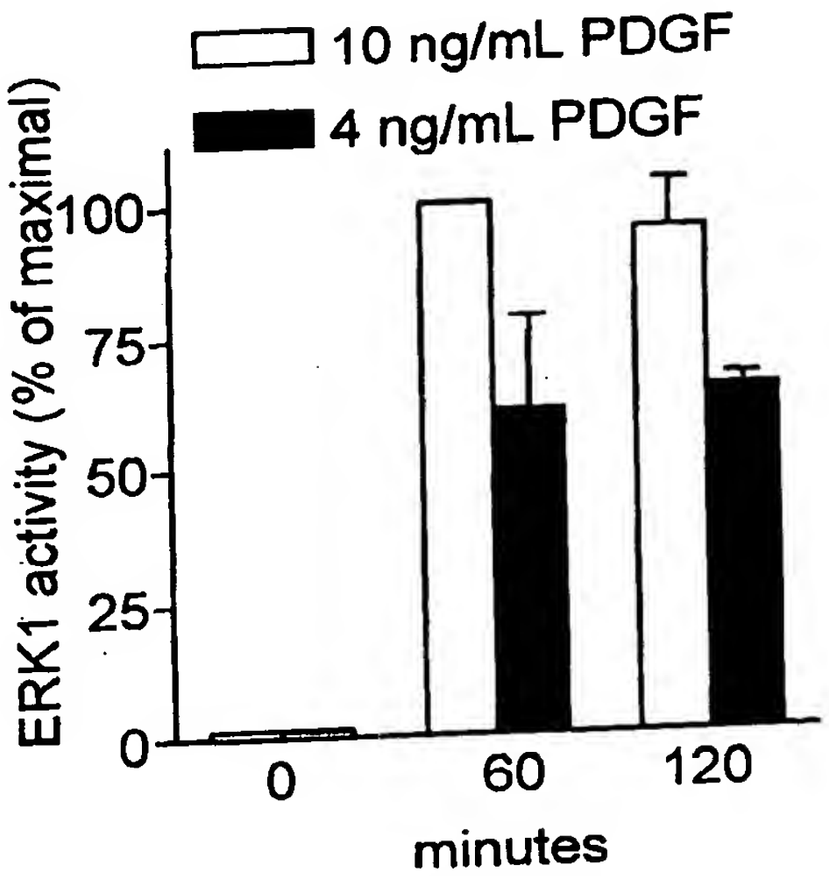

α Egr-1
α c-Fos
α Fra-2
α Fra-1
α ERK
hours: 0 2 3 4 2 3 4 2 3 4 2 3 4
PDGF: 1.0 ng/mL 6 ng/mL 5 ng/mL 4 ng/mL

Figure 21


A
10 ng/mL PDGF
4 ng/mL PDGF
ERK1 activity (% of maximal)
100
75
50
25
0
0
60
120
minutes

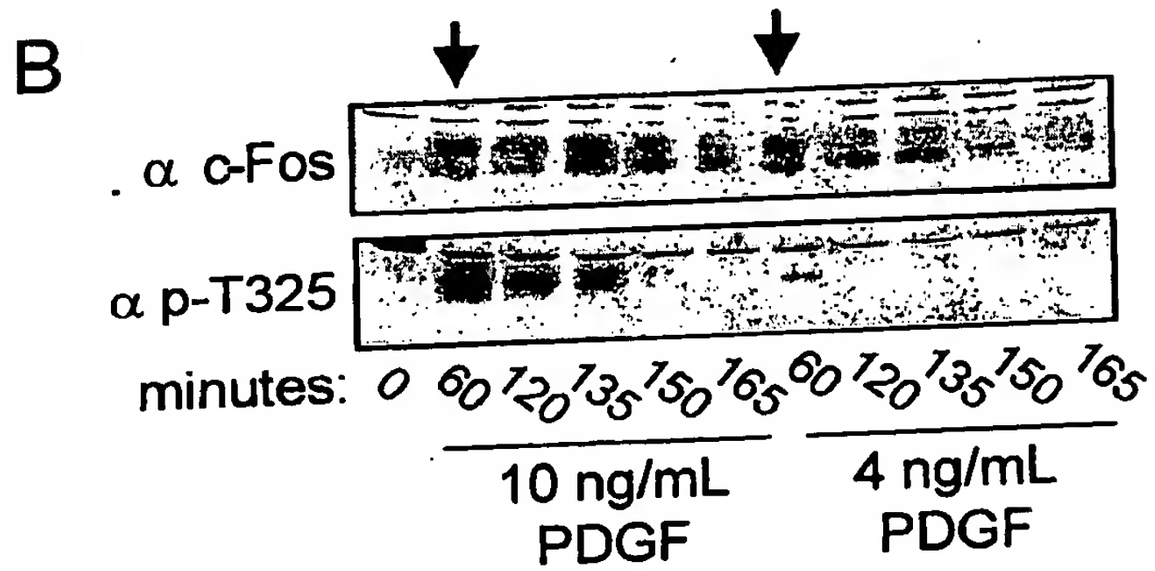

B
α c-Fos
α p-T325
minutes: 0 60 120 135 150 165 60 120 135 150 165
10 ng/mL PDGF
4 ng/mL PDGF

Figure 22

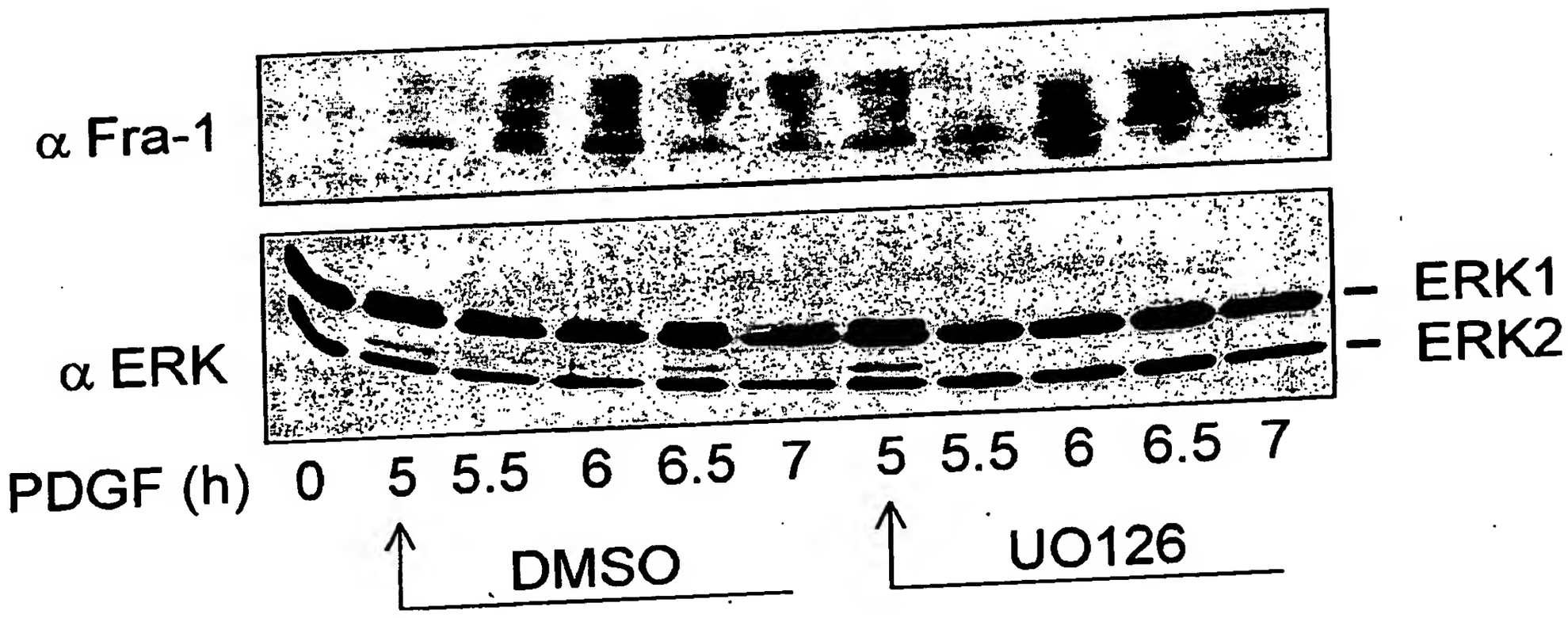


Figure 23

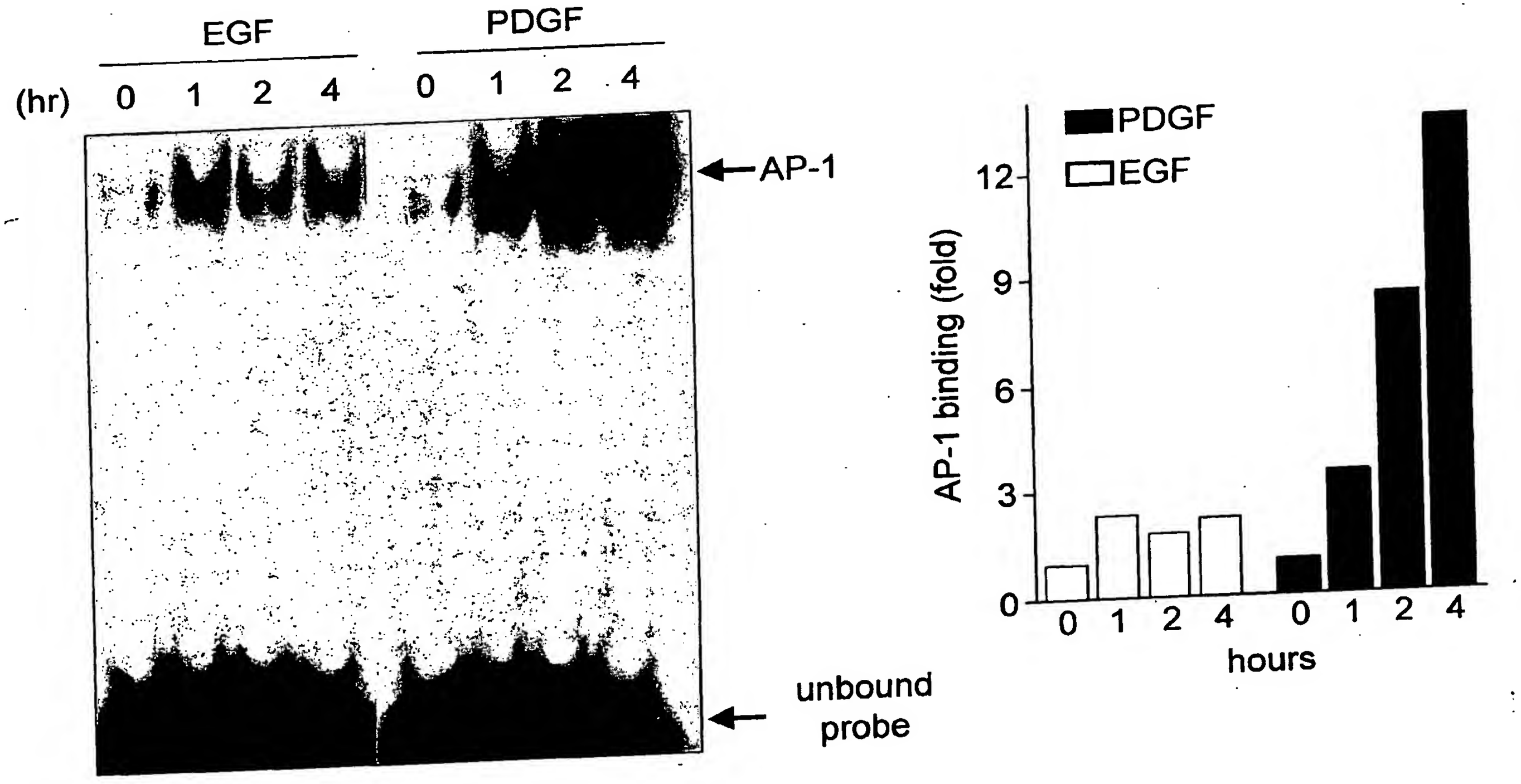
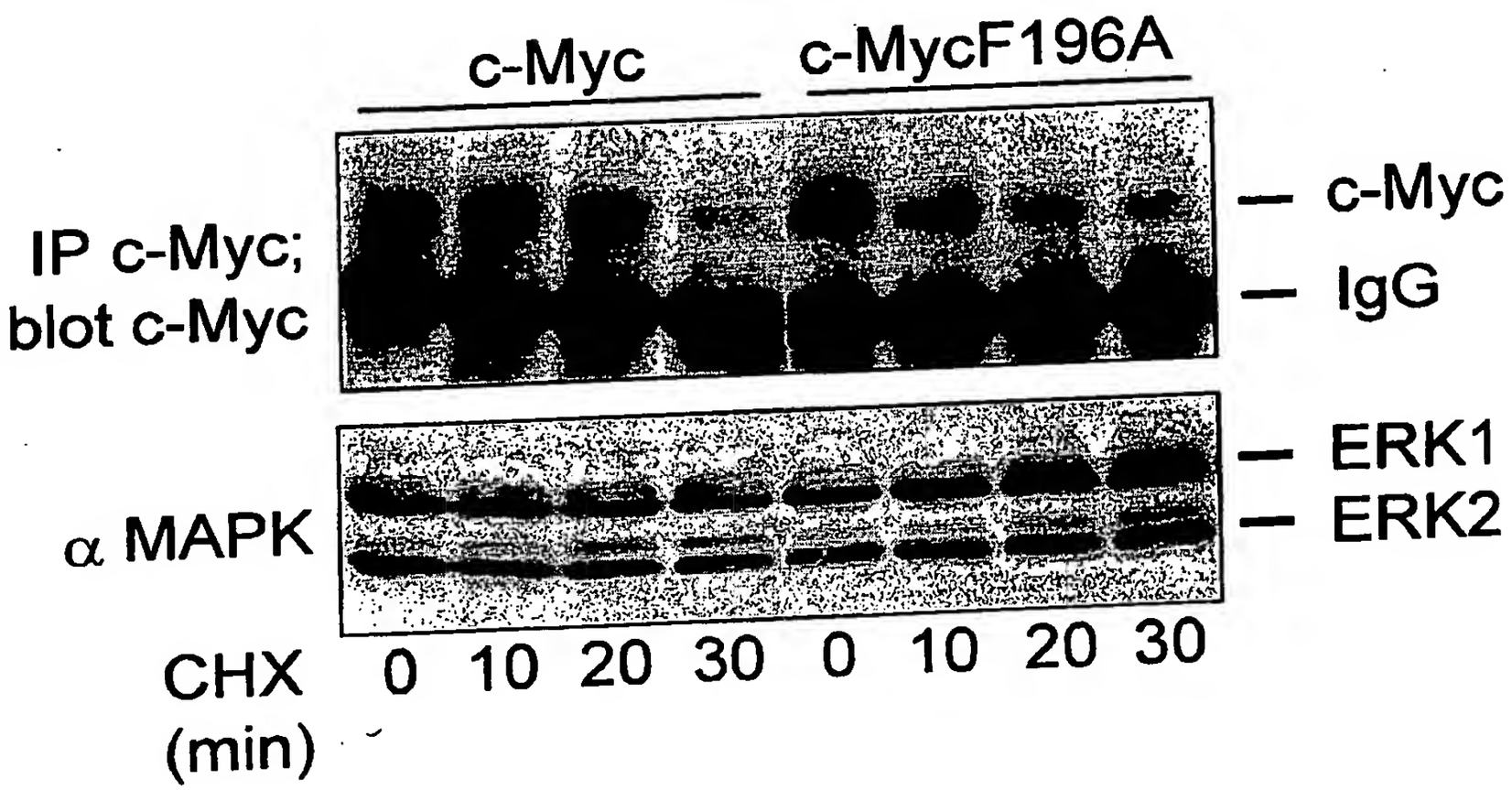

EGF
PDGF
(hr) 0 1 2 4 0 1 2 4
AP-1
unbound probe
PDGF
EGF
AP-1 binding (fold)
0
3
6
9
12
0 1 2 4 0 1 2 4
hours

Figure 24


c-Myc
c-MycF196A
IP c-Myc;
blot c-Myc
c-Myc
IgG
α MAPK
ERK1
ERK2
CHX 0 10 20 30 0 10 20 30
(min)

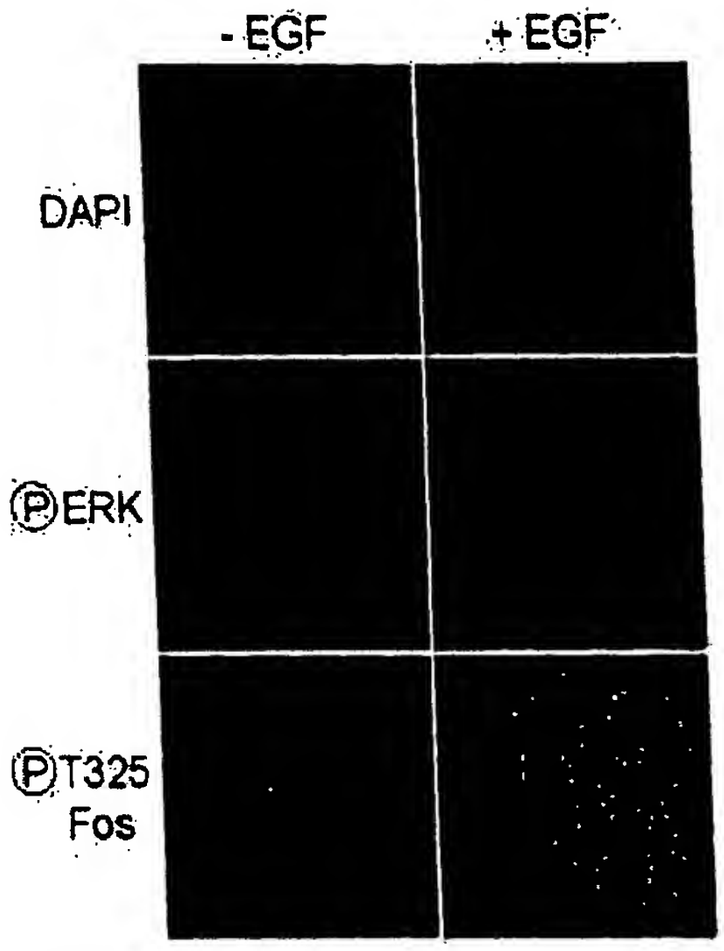


FIGURE 25